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(71) Applicant *(for all designated States except US)*: NORTRAN PHARMACEUTICALS, INC. [CA/CA]; 3650 Wesbrook Mall, Vancouver, British Columbia V6S 2L2 (CA).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: HAYES, Eric, S. [US/CA]; #101, 1234 Fort Street, Victoria, British Columbia V8V 3L2 (CA).

(74) Agents: PARKER, David, W. et al.; Seed and Berry LLP, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

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(54) Title: SEROTONIN LIGANDS AS PRO-ERECTILE COMPOUNDS

(57) Abstract

Compounds, and compositions including the compounds, are effective to treat or prevent sexual dysfunction, (by, for example, providing a pro-erectile function), when those compounds display selective serotonin (5HT) receptor activity. In one aspect, the compound is a 5HT2c agonist and a 5HT2a antagonist. In another aspect, the compound is a 5HT2c agonist, a 5HT2a antagonist, and a 5HT1a agonist (weak).

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SEROTONIN LIGANDS AS PRO-ERECTILE COMPOUNDS

TECHNICAL FIELD

The present invention is generally directed to therapeutic agents, and in particular to agents that enhance sexual performance, such as pro-erectile compounds, and is also directed to composition containing these agents, and is also directed to therapeutic agents and composition that are characterized by one or more receptor activity profiles, and to methods of screening and identifying therapeutic agents according to their receptor activity profiles, and methods of treatment that employ such therapeutic agents and compositions. The therapeutic agent may be employed to enhance sexual performance, as pro-libido agents and/or for the treatment and/or prevention of sexual dysfunction in males and/or females.

BACKGROUND OF THE INVENTION

At the present time there is a wide variety of pharmacological agents used and/or reportedly useful as pro-libido agents and/or for the treatment of sexual dysfunction. Some examples include: serotonin receptor agonists and antagonists (*see, e.g.*, EP 385,658; WO 94/15,920; GB 2,248,449; and GB 2,276,165), dopamine receptor agonists (*see, e.g.*, WO 93/23,035; WO 94/21,608; Pomerantz S. M., Pharmacol. *Biochem. Behav. 39*:123-128, 1991; and Ferrari F. et al. *Psychopharmacology 113*:172-176, 1993); adrenergic receptor agonists (*see, e.g.*, WO 95/13,072; EP 611,248; US 5,229,387; and WO 92/11,851); inhibitors of phoshodiesterase (*see, e.g.*, DE 4,338,948; and WO 94/28,902); histamine receptor agonists (*see, e.g.*, US 4,013,659; US 4,126,670; US 4,767,778; WO 91/17,146; US 5,047,418; and EP 0,458,661); neuropeptide Y antagonists (*see, e.g.*, WO 95/00,161); angiotensin II receptor antagonists (*see, e.g.*, EP 577,025); cholinesterase inhibitors (*see, e.g.*, US 5,177,070; and US 4,633,318); combinations of agents with the different types of biological activity (*see, e.g.*, US 5,145,852; and WO 95/05,188); derivatives of vasoactive intestinal peptide (*see, e.g.*, US 5,147,855; EP 540,969; and EP 463,450); prostaglandins (*see, e.g.*, WO 93/00,894; and EP 459,3770); antidepressants

and antipsychotics (*see, e.g.*, US 4,931,445; GB 2,448,449; and Naganuma et al: *Clin. Exp. Pharm. Physiol. 20*:177-183, 1993); nitric oxide donors (*see, e.g.*, WO 92/21,346; DE 4,305,881; DE 4,212,582; and WO 94/16,729); calcitonin gene related peptide (*see, e.g.*, Steif, C. G. et al., *Urology, 41*:397-400, 1993); and androgens (*see, e.g.*, JP 06,211,675; HU 62,473; and WO 94/16,709).

Many or all of these pharmacological agents are associated with adverse effects, some examples of which are quoted below. Dopamine receptor agonists may aggravate schizophrenia or induce it *de novo* in some patients. Serotonin receptor agonists are capable of producing an effect that has been termed "serotonin syndrome" (Glennon, R.A. *J. Med. Chem.* 30:1-9,1987). This latter effect has been thoroughly investigated in animals (Peroutka, S. J. *Science 212*:827-829, 1981; Goddwin G. M. et al., *Br. J. Pharmacol. 84*:743-753, 1985; and Tricklebank, M. D., *Eur. J. Pharmac. 117*:15-24, 1985) and manifests itself in, for example, head twitches, "wet dog shakes", forepaw treading, flat body posture, hind limb abduction, Straub tail and yawning. Histamine receptor agonists may induce central nervous system dysfunction and adverse effects in the endocrine system. Smooth muscle relaxants (such as papaverine) may induce pain, echytomosis and occasional episodes of priapism. α-Adrenoreceptor blockers administered systemically have been reported to induce priapism characterized by a persistent erection that cannot be relieved by sexual intercourse or masturbation (Kaisary, A.V. et al., *Br. J. Urol. 68*:227, 1986).

Accordingly there is a need in the art to identify new pharmacological agents, compositions and/or treatments which are useful as pro-libido agents and/or are useful in the treatment and/or prevention of sexual dysfunction in males or females, and/or can enhance a patient's sexual performance. The present invention fulfills these needs and further provides related advantages.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides for the use of a compound, or a combination of two or more compounds, for the manufacture of a medicament. In one embodiment, the compound or two or more compounds can

occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}. In another embodiment, the compound or two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In another embodiment, the compound or two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In another embodiment, the compound or two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}. Such a medicament as described above may be used to achieve one or more of the following goals: treating and/or preventing sexual dysfunction in a patient; increasing the libido of a male or female patient; and enhancing the sexual performance of a male or female patient.

Thus, the present invention provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient. In the foregoing, the sexual dysfunction may be, for example, male erectile dysfunction; impotence; or female sexual arousal disorder and/or female inhibited orgasm.

Thus, the present invention provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for increasing the libido of a male or female patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-

HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for increasing the libido of a male or female patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for increasing the libido of a male or female patient; the use of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for increasing the libido of a male or female patient.

Thus, the present invention provides for the use of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient; the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient. In the foregoing, enhancing the sexual performance of the male or female patient may provide, for example, a pro-erectile response in the patient.

The present invention further provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; or for the manufacture of a medicament for increasing the libido of a male or female patient, or for the manufacture of a medicament for enhancing the sexual performance of a male or female patient. In one embodiment, the compound or the combination of two or more

compounds, provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

The present invention further provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; or for the manufacture of a medicament for increasing the libido of a male or female patient, or for the manufacture of a medicament for enhancing the sexual performance of a male or female patient. In one embodiment, the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

The present invention further provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; or for the manufacture of a medicament for increasing the libido of a male or female patient, or for the manufacture of a medicament for enhancing the sexual performance of a male or female patient. In one embodiment, the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

The present invention further provides for the use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient; or for the manufacture of a medicament for increasing the libido of a male or female patient, or for the manufacture of a medicament for enhancing the sexual performance of a male or female patient. In one embodiment, the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

In one embodiment, the compound or the combination of two or more compounds may be formulated for oral administration. In another embodiment, the compound or the combination of two or more compounds is formulated for topical administration. In another embodiment, the compound or the combination of two or more compounds is formulated for direct injection. In another embodiment, the compound or the combination of two or more compounds is formulated for one of intrameatal, intracavernous or intraurethral administration. In another embodiment, the compound or the combination of two or more compounds is formulated as a tablet with a disintegration time of less than one hour.

In an optional embodiment of the above-described uses of the present invention, the compound or combination of two or more compounds does not interact with the alpha-adrenoceptors and furthermore optionally does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor. In another optional embodiment of the above-described uses of the present invention, the compound or combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

In another aspect, the present invention provides pharmaceutical compositions. The compositions comprise a pharmaceutically acceptable carrier or diluent and a compound or a combination of two or more compounds having the properties described above. In one embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}. The pharmaceutical composition may be in the form of a tablet for oral administration, where the tablet preferably has a disintegration time of less than one hour.

In another aspect, the present invention provides for a method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound, or a therapeutically effective dose of a combination of two or more compounds, or a therapeutically effective dose of a pharmaceutical composition comprising a compound, or a therapeutically effective does of a pharmaceutical composition comprising a combination of two or more compounds. In one embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

The therapeutically effective dose may achieve, for example, treatment and/or prevention of sexual dysfunction, where exemplary sexual dysfunction include male erectile dysfunction, impotence, or female sexual arousal disorder and/or female inhibited orgasm. The therapeutically effective does may achieve, for example, an increase in the libido of a male or female patient. The therapeutically effective does may achieve, for example, an enhancement in the sexual performance of a male or female patient.

The present invention also provides a method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound, or a therapeutically effective dose of a combination of two or more compounds, or a therapeutically effective dose of a pharmaceutical composition comprising a compound, or a therapeutically effective dose of a pharmaceutical composition comprising a combination of two or more compounds. In one embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

The present invention also provides a method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound, or a therapeutically effective dose of a combination of two or more compounds, or a therapeutically effective dose of a pharmaceutical composition comprising a compound, or a therapeutically effective dose of a pharmaceutical composition comprising a combination of two or more compounds. In one embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In another embodiment, the compound or combination of two or more compounds can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}. In one embodiment, the dose provides a pro-erectile response in the patient.

In another aspect, the present invention provides a method for treating or preventing sexual dysfunction in a patient, or increasing the libido of a male or female patient, or enhancing the sexual performance of a male or female patient. The method comprises administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}. In a preferred embodiment, the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

In another aspect, the present invention provides a method for treating or preventing sexual dysfunction in a patient, or increasing the libido of a male or female patient, or enhancing the sexual performance of a male or female patient. The method comprises administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3. In a preferred embodiment, the compound or combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

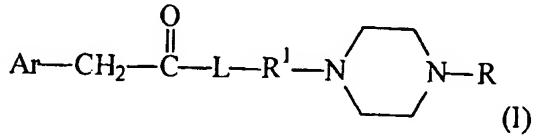
In another aspect, the present invention provides a method for treating or preventing sexual dysfunction in a patient, or increasing the libido of a male or female patient, or enhancing the sexual performance of a male or female patient. The method comprises administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}. In a preferred embodiment, the compound or combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

In another aspect, the present invention provides a method for treating or preventing sexual dysfunction in a patient, or increasing the libido of a male or female patient, or enhancing the sexual performance of a male or female patient. The method comprises administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}. In a preferred embodiment, the compound or combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

In one aspect, the present invention provides a method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A} in a patient, comprising

administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I), while in a preferred embodiment, the compound or the combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor. In another aspect, the present invention provides a method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3 in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I), while in a preferred embodiment, the compound or the combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor. In another aspect, the present invention provides a method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A} in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I), while in a preferred embodiment, the compound or combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor. In another aspect, the present invention provides a method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A} in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I), while in a preferred embodiment, the compound or combination of two or more compounds provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

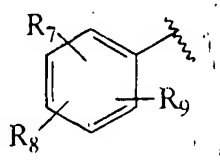
In the above methods, compounds of formula (I) have the structure:



(I)

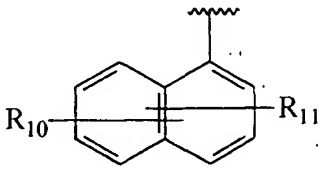
including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):



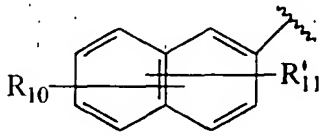
(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



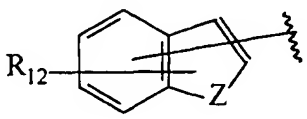
(III)

and



(IV)

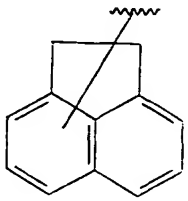
where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



(V)

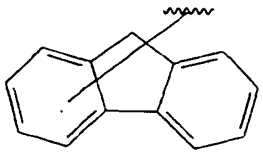
where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl,

trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-$CH_2C(O)$-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



(VI)

and



(VII);

L is selected from the group of a direct bond, O, NH, and $N(C_1$-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

In an optional embodiment of the above-described methods of the present invention, the compound or combination of two or more compounds does not interact with the alpha-adrenoceptors, and furthermore, optionally, does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor. In another optional embodiment of the above-described methods of the present invention, the compound or combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor. Also, in any of the above-described methods, the administration may be by oral administration, topical administration, direct injection, or one of intrameatal, intracavernous or intraurethral administration.

In another aspect, the present invention provides a method for screening test compounds for pro-erectile activity. The method comprises:

(a) contacting a test compound with a serotonin 5-HT_{2C} receptor, and measuring the binding of the compound to the 5-HT_{2C} receptor; and

(b) contacting the test compound with a serotonin 5-HT_{2A} receptor, and measuring the binding of the test compound to the 5-HT_{2A} receptor.

In an optional embodiment, the screening method additionally comprises;

(c) contacting the test compound with a serotonin 5-HT_3 receptor, and measuring the binding of the test compound to the 5-HT_3 receptor.

In one embodiment of the screening methods of the present invention the binding is measured by the %inhibition by the test compound on the binding of specific radioligand to the respective 5-HT subtype receptors (*e.g.*, [3H]-Ketanserin for 5-HT_{2A}; [3H]-Mesulergine for 5-HT_{2C}; and [3H]-GR65630 for 5-HT_3). In one embodiment, the %inhibition is at least 30% and preferably 50% or more. In another embodiment, the compound is a piperazine derivative. The method may use a purified receptor.

These and other aspects and embodiments of the present invention will become evident upon reference to the following detailed description and attached Figures. The aspects and embodiments described herein may be combined, to the extent they are not mutually exclusive.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the effects of Compound 1(4.0-64.0 mg/kg; i.p.) on erection (■; solid line) and latency to first erection (●; dashed line) in male rats compared to saline controls (C; empty symbols). Proportions above the data points indicate the number of test animals exhibiting erection at a given dose. Values represent mean ± SEM for 10 test animals. An asterisk indicates a significant difference versus saline treated animals as determined by ANOVA ($p< 0.05$).

Figure 2 is a graph showing the effects of Compound 1(2.0-64.0 mg/kg; ●, solid line), RSD1026 (2.0-32.0 mg/kg; ■, dashed line) and RSD1052 (2.0-32.0 mg/kg; ◆, stippled line) on erections in isolated male rats compared to saline controls (C; Δ). Values represent mean ± SEM for 10 test animals. An asterisk

indicates a significant difference versus saline treated animals as determined by ANOVA ($p < 0.05$).

Figure 3 is a graph showing the effects of m-Chlorophenylpiperazine (m-CPP; 0.2-3.12 mg/kg; ●, solid line). Trifluoromethylphenylpiperazine (TFMPP; 0.2-3.12 mg/kg; ■, dashed line) and 1-Naphthylpiperazine (1-NP; 0.2-3.12 mg/kg; ◆, stippled line) on erections in isolated male rats compared to saline controls (Δ). Values represent mean ± SEM for 10 test animals. An asterisk indicates a significant difference versus saline treated animals as determined by ANOVA ($p < 0.05$).

Figure 4 is a graph showing the effects of Cpd. 4 (0.1-10 mg/kg; ●, solid line), Cpd. 5 (0.1-10 mg/kg; ■, dashed line) and Cpd. 6 (0.1-10 mg/kg; ◆, stippled line) on erections in isolated male rats compared to saline controls (Δ). Values represent mean ± SEM for 10 test animals. An asterisk indicates a significant difference versus saline treated animals as determined by ANOVA ($p < 0.05$).

Figure 5 is a graph showing the effects of chronic high (Rit; 3.0 mg/kg; diagonal striped column) and low (Rit; 0.3 mg/kg; cross hatched column) doses of ritanserin on erections induced by Compound 1 (992; 16 mg/kg; horizontal striped column). Values represent the mean ± SEM for n=5 animals. An asterisk indicates a significant difference from control animals which received only saline (Sal; 1.0 mg/kg; solid column) whereas a + indicates a significant difference from animals receiving vehicle (Veh; 100% DMSO) prior to Compound 1 ($p < 0.05$ by ANOVA with Dunnett's test for multiple comparisons).

Figure 6 is a graph showing the effects of chronic high (Min; 3.0 mg/kg; diagonal striped column) and low (Min; 0.3 mg/kg; vertical striped column) doses of mianserin on erections induced by Compound 1 (992. 1; 16 mg/kg; solid column). Values represent the mean ± SEM for n=5 animals. An asterisk indicates a significant difference from control animals which received only saline (1.0 mg/kg; blank column) whereas a + indicates a significant difference from animals receiving vehicle (Veh; saline) prior to Compound 1 ($p < 0.05$ by ANOVA with Dunnett's test for multiple comparisons).

Figure 7 is a graph showing the effects of NAN-190 (NAN; 0.2-2.0 mg/kg; i.p.; striped bars) on erections in male rats elicited by Compound 1 (992; 16 mg/kg; i.p.; cross hatched bars). Control rats (solid bars) received two doses of saline (Sal; 1.0 ml/kg; i.p.). Data are expressed as mean ± SEM for n=5 animals. An asterisk indicates significant difference compared to control rats whereas a + indicates a significant difference from animals receiving vehicle (saline) and Compound 1 ($p < 0.05$ by ANOVA with Dunnett's test for multiple comparisons).

Figure 8 is a graph showing the effects of saline (hatched columns), Compound 1 (solid columns), m-CPP (diagonal striped columns) and TFMPP (vertical striped columns) on erection (left side) and ejaculation (right side) in rats observed in pairs. Values represent mean ± SEM for 15 test animals per group. An asterisk indicates significant difference compared to saline treated animals ($p < 0.05$; ANOVA with Dunnett's test for multiple comparisons).

Figure 9 is a graph showing the effects of saline (hatched columns), Compound 1 (solid columns), m-CPP (diagonal striped columns) and TFMPP (vertical striped columns) on rearing (left side) and movement (right side) in rats observed in pairs. Values represent mean ± SEM for 15 test animals per group. An asterisk indicates significant difference compared to saline treated animals ($p < 0.05$; ANOVA with Dunnett's test for multiple comparisons).

Figure 10 is a graph showing the effects of saline (hatched columns), Compound 1 (solid columns), m-CPP (diagonal striped columns) and TFMPP (vertical striped columns) on penile (left side) and non-penile (right side) grooming in rats observed in pairs. Values represent mean ± SEM for 15 test animals per group. An asterisk indicates significant difference compared to saline treated animals ($p < 0.05$; ANOVA with Dunnett's test for multiple comparisons).

Figure 11 is a graph showing the lack of effects of Compound 1 on KCl mediated contraction of rat vascular smooth muscle (aorta). Data are expressed as the percent maximal response to the KCl in the presence of saline (+, solid line; n=6) or a high concentration of Compound 1 (10^{-4} M; ●; dashed line; n=6). Values represent the mean ± SEM for the number of tissues indicated by n.

Figure 12 is a graph showing the lack of effect of Compound 1 on NA mediated contraction of rat vascular smooth muscle (aorta). Data are expressed as the percent maximal response to NA in the presence of saline (÷, n=4) or increasing concentrations of Compound 1 (10^{-5} M, ●; 3 x 10^{-5} M, ■; 10^{-4} M, ◆; n=4). Values represent the mean ± SEM for the number of tissues indicated by n.

Figure 13 is a graph showing the effects of Compound 1 on 5-HT mediated contraction of isolated rat aorta. Data are expressed as the percent maximal response to 5-HT in the presence of saline (÷, n=7) or increasing concentrations of Compound 1 (3 x 10^{-6} M, ●; 10^{-5} M, ■; 3 x 10^{-4} M, ◆; 10^{-4} M, Δ; n=6-7). Values represent the mean ± SEM for the number of tissues indicated by n.

Figure 14 is a graph showing the effects of Compound 1 on 5-HT mediated contraction of rat basilar artery. Data are expressed as the percent maximal response to 5-HT in the presence of saline (÷, n=8) or increasing concentrations of Compound 1 (10^{-5} M, ●; 10^{-4} M, ■; n=4-6). Values represent the mean ± SEM for the number of tissues indicated by n.

Figure 15 is a graph showing the contractile effects of 5-HT (+), m-CPP (◆) and Compound 1 (●) on rat stomach fundus. Values represent the mean ± SEM for n = 12 strips from 6 animals.

Figure 16 is a graph showing the effects of increasing doses of Compound 1 (10^{-8} M (◆), 10^{-6} M (■), 10^{-4} M (●)) and 1-naphthylpiperazine (3 x 10^{-9} M (Δ), 3 x 10^{-7} M (∇)) and saline (+) on 5-HT mediated contraction of the isolated rat stomach fundus. Data are expressed as the mean ± SEM for 4-12 strips from 2-6 animals.

Figure 17 is a graph showing the effects of increasing doses of Compound 1 (92; 1.0-64.0 mg/kg; diagonal striped columns) on changes in core temperature in rats. Saline (1.0 ml/kg) treated and 8-OHDPAT (0.65 mg/kg) treated control animals are represented by solid and cross hatched columns respectively. Animals receiving Compound 1 at a dose of 64.0 mg/kg in the presence of saline are represented by a vertical striped column. Pre-treatment and challenge are separated by a forward slash for saline (Sal), 8-OHDPAT (D) and Compound 1 (92(dose in

parentheses)). Ambient temperature ranged from 27.4-29.0 ° C. Values are expressed as mean ± SEM for 5 animals per test group. An asterisk indicates a significant difference from saline treated controls whereas a + indicates a significant difference from 8-OHDPAT treated controls ($p < 0.05$ ANOVA and Dunnett's test for multiple comparisons).

Figure 18 is a graph showing the effects of increasing doses of Compound 1 (92; 2.0-32.0 mg/kg; diagonal striped columns) on changes in core temperature in rats. Saline (1.0 ml/kg) treated and 8-OHDPAT (0.65 mg/kg) treated control animals are represented by solid and cross hatched columns respectively. Animals receiving Compound 1 at a dose of 32.0 mg/kg prior to 8-OHDPAT (0.65 mg/kg) are represented by a vertical striped column. Pre-treatment and challenge are separated by a forward slash for saline (Sal). 8-OHDPAT (D) and Compound 1 (92(dose in parentheses)). Ambient temperature ranged from 27.0-29.0 ° C. Values are expressed as mean ± SEM for 5 animals per test group. An asterisk indicates a significant difference from saline treated controls ($p < 0.05$ ANOVA and Dunnett's test for multiple comparisons).

Figure 19 is a graph showing the inhibitory effects of Compound 1 (992; (2.0-32.0 mg/kg)); diagonal striped column) and ketanserin (Ket (1.0 mg/kg); horizontal striped column) on DOI (DOI (2.5 mg/kg); cross-hatched column) induced head-twitch responses. Head-twitch responses in animals receiving only saline are indicated by a solid column (C). Values represent the mean ± SEM for the number of head-twitches scored for 10 minutes in 5 animals. An asterisk indicates a significant difference from saline controls whereas a + indicates a significant difference from DOI treated animals receiving saline ($p < 0.05$; ANOVA with Dunnett's test for multiple comparisons).

Figure 20 is a graph showing the effects of increasing doses of Compound 1 (●), quipazine (Δ), m-CPP (■), TFMPP(◆) and saline (∇)on bradycardia induced by i.v administration of serotonin. Data are expressed as the reduction in heart rate in beats per minute after 5-HT administration. C indicates pre-drug responses to test doses of 5-HT. Values represent the mean ± SEM for 6

experiments. An asterisk indicates a significant reduction the bradycardic response to 5-HT compared to pre-drug ($p < 0.05$; ANOVA).

DETAILED DESCRIPTION OF THE INVENTION

An understanding of the present invention may be aided by reference to the following definitions and explanation of conventions used herein.

Definitions and Conventions

ABBREVIATIONS	
Alpha	(α)
Analysis of variance	(ANOVA)
atrio-ventricular	(A-V)
Beta	(β)
Cavernous nerve	(CN)
Cavernous nerve activity	(CNA)
Central nervous system	(CNS)
Concentration producing 50 % maximal effect	(EC_{50})
Concentration-response curves	(CRC's)
Corpus cavernosal	(CC)
Cyclic adenosine monophosphate	(cAMP)
Cyclic guanosine monophosphate	(cGMP)
Degrees Celsius	(°C)
Dose producing 50 % maximal effect	(ED_{50})
Dose producing -50 % maximal effect	(ID_{50})
Electrical field stimulated	(EFS)
electrocardiogram	(ECG)
Established α level	(p)
Frequency producing 50 % maximal effect	(EF_{50})
Frequency-response curves	(FRC's)
Gas mixture (95 %O_2, 5 % $C0_2$)	(Carbogen®)
gram	(g)
Gauge	(G)
Hertz	(Hz)
Inhibitory G-protein	(G_i/G_o)
Inositoltriphosphate	(IP_3)
International units	(i.u.)
Intraperitoneal	(i.p.)
Intracavernosal pressure	(ICP)
Intracerebroventricular	(ICV)
Kilogram	(kg)

ABBREVIATIONS	
Locus coeruleus	(LC)
Medial pre-optic area	(MPOA)
messenger ribonucleic acid	(mRNA)
Micro	(μ)
Milligram	(mg)
Milliliter	(ml)
Millisecond	(msec)
Minute	(min)
Molar	(M)
negative logarithm of EC50	(pD_2)
Non-adrenergic non-cholinergic	(NANC)
Omega	(ω)
Paraventricular nucleus	(PVN)
Polyethylene	(PE)
Rhythm Search Developments	(RSD)
Serotonin selective re-uptake inhibitor	(SSRI)
Sprague-Dawley	(SD)
Standard error of the mean	(SEM)
Stimulatory G-protein	($G_{s/\alpha q}$)
subcutaneous	(s.c.)
ventral tegmental area	(VTA)
Volt	(V)

CHEMICAL NAMES	
[^{3}H]	Tritiated hydrogen ion
1-PBG	1-phenylbiguanide
5,7-DHT	5,7-dihydroxytryptamine
5-HT	Serotonin
5-MeODMT	5-methoxy-N, N-dimethyltryptamine
5-MeOT	5-methoxytryptamine
8-OHDPAT	8-hydroxy-2-(di-n-propylamino)tetralin
Ach	Acetylcholine
BRL 46470A	(endo-N-(8-methyl-8-azabicyclo [3.2.1]oct-3-yl)2,3-dihydro-3,3dimethyl-indole-1-carboxamide,hydrochloride)
$CaCl_2$	Calcium chloride
CGS 12066B	[7-trifluormethyl-4-(4-methyl-1-piperazonyl)-pyrrolol-[1-2-a]quinoxaline dimaleate]
CO_2	Carbon dioxide
CP-93,129	3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one
DHPTHP	dihydroxyphenyl-tetrahydrothieneopyridine
d-LSD	d-Lysergic acid diethylamide
DMSO	Dimethylsulfoxide
DOB	2,5-Dimethoxy-4-bromoamphetamine

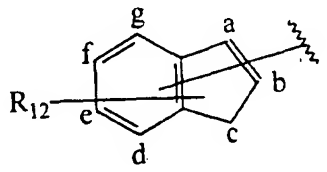
CHEMICAL NAMES	
DOI	(+-)-2,5-dimethoxy-4-iodophenyl-2-aminopropane
DOM	2,5-dimethoxy-4-methylamphetamine
DSP_4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine
DTG	1,3-di-o-tolylguanidine
ET	Endothelin
FR121196	N-(4-Acetyl- 1 -piperazinyl)-4-fluorobenzenesulfonamide
GABA	Gamma amino butyric acid
GR 113808	[[1-[2-methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1 H-indole-3-carboxylate]
GR127935	2'-methyl-4'-(5-methyl[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]amide
GR46611	4-[3-[2-(N,N-dimethylamino)ethyl]-1H-indol-3-yl]-N-(p-methoxybenzyl) acrylamide
ICS 205,930	[(3 alpha-tropanyl)-1H-indol-3-carboxylic acid ester]
isamoltane	CGP 361A. 1-(2-(1-pyrrolyl)-phenoxy)-3-isopropylamino-2-propranol
K^+	Potassium
KCl	Potassium chloride
KH_2PO_4	Potassium phosphate
L-5-HTP	L-5-hydroxytryptophan
L-NAME	NG-nitro-L-arginine methyl ester
MDL 72222	[(1 alpha H, 3alpha, 5 alpha H)-tripan-3-yl-3,5-dichlorobenzoate
MDL100,907	(+/-)2,3-dimethoxyphenyl- 1 -[2-(4-piperidine)-methanol]
$MgSO_4$	Magnesium sulfate
MK 212	[6-chloro-2-(1-piperazinyl)pyrazine]
NA	Noradrenaline
$NaHCO_3$	Sodium bicarbonate
NO	Nitric oxide
NPY	Neuropeptide Y
NTG	Nitroglycerine
PCA	p-chloroamphetamine
pCPA	p-chlorophenylalanine
PGEI	Prostaglandin El
quipazine	2-(1-piperazinyl)quinoline maleate
RO 60-0175	(S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine) fumarate
RS- 102221	(a benzenesulfonamide of 8-[5-(5-amino-2,4-dimethoxyphenyl)5-oxopentyl]-1,3,8triazaspiro[4.5]decane-2,4-dione
RU 24969	(5-methoxy-3-(1,2,3,6-tetrahydro-4- pyridinyl)-1H indole
SB 200,646	(1-(1-methylindol-5-yl)-3-(3-pyridyl) urea)
SB 206,553	(5 methyl-1-(3-pyridil-carbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3 -f]indole)
SC53116	(1S-cis)-4-Amino-5-chloro-N-[(hexahydro-1H-pyrrolizin- 1 -yl)methyl]-2-methoxy-benzamide
SCH23390	7-chloro-8-hydroxy-3-methyl- 1 -phenyl-2,3,4,5-tetrahydro-1H-3-benzaz epine maleate
SP	Substance P

CHEMICAL NAMES	
VIP	Vasoactive intestinal polypeptide
WAY100635	N-[2-[4-(2-methoxypheny])-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride

In the formulae depicted herein, a bond to a substituent and/or a bond that links a molecular fragment to the remainder of a compound may be shown as intersecting one or more bonds in a ring structure. This indicates that the bond may be attached to any one of the atoms that constitutes the ring structure, so long as a hydrogen atom could otherwise be present at that atom. Where no particular substituent(s) is identified for a particular position in a structure, then hydrogen(s) is present at that position.

In those instances where the invention specifies that a non-aromatic ring is substituted with more than one R group, and those R groups are shown connected to the non-aromatic ring with bonds that bisect ring bonds, then the R groups may be present at different atoms of the ring, or on the same atom of the ring, so long as that atom could otherwise be substituted with a hydrogen atom.

Likewise, where the invention specifies compounds containing the Ar-$CH_2C(O)$-L- group where Ar equals the group (V)



(V)

the invention is intended to encompass compounds wherein -$CH_2C(O)$-L- is joined through CH_2 to the Ar group (V) at any atom that forms the group (V) so long as that atom of group (V) could otherwise be substituted with a hydrogen atom. Thus, there are seven positions (identified with the letters "a" through "g") in structure (V) where the -$CH_2C(O)$-L- group could be attached, and it is attached at one of those seven positions. The R_{12} group would occupy one and only one of the remaining six positions, and hydrogen atoms would be present in each of the five remaining positions.

The compounds of the present invention may contain two or more asymmetric carbon atoms and thus exist as enantiomers and diastereomers. Unless otherwise noted, the present invention includes all enantiomeric and diastereomeric forms of the compounds of the invention. Pure stereoisomers, mixtures of enantiomers and/or diastereomers, and mixtures of different compounds of the invention are included within the present invention. Thus, compounds of the present invention may occur as racemates, racemic mixtures and as individual diastereomers, or enantiomers with all isomeric forms being included in the present invention. A racemate or racemic mixture does not imply only a 50:50 mixture of stereoisomers. The compounds of formula (I) or formula (XX) may also exist in tautomeric forms and the invention includes both mixtures and separate individual tautomers.

The phrase "independently at each occurrence" is intended to mean (i) when any variable occurs more than one time in a compound of the invention, the definition of that variable at each occurrence is independent of its definition at every other occurrence; and (ii) the identity of any one of two different variables (*e.g.*, R_1 within the set R_1 and R_2) is selected without regard the identity of the other member of the set. However, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

In accordance with the present invention and as used herein, the following terms are defined to have following meanings, unless explicitly stated otherwise:

"Acid addition salts" refers to those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid and the like.

"Acyl" refers to branched or unbranched hydrocarbon fragments terminated by a carbonyl -(C=O)- group containing the specified number of carbon atoms. Examples include acetyl [$CH_3C(=O)$-, a C_2acyl] and propionyl [$CH_3CH_2(C=O)$-, a C_3acyl].

"Alkanoyloxy" refers to an ester substituent wherein the non-carbonyl oxygen is the point of attachment to the molecule. Examples include propanoyloxy [$(CH_3CH_2(C=O)-O$-, a C_3alkanoyloxy] and ethanoyloxy [$CH_3(C=O)-O$-, a C_2alkanoyloxy].

"Alkoxy" refers to an O-atom substituted by an alkyl group, for example, methoxy [$-OCH_3$, a C_1alkoxy].

"Alkoxyalkyl" refers to an alkylene group substituted with an alkoxy group. For example, methoxyethyl [$CH_3OCH_2CH_2$-] and ethoxymethyl ($CH_3CH_2OCH_2$-] are both C_3alkoxyalkyl groups.

"Alkoxycarbonyl" refers to an ester substituent wherein the carbonyl carbon is the point of attachment to the molecule. Examples include ethoxycarbonyl [$CH_3CH_2O(C=O)$-, a C_3alkoxycarbonyl] and methoxycarbonyl [$CH_3O(C=O)$-, a C_2alkoxycarbonyl].

"Alkyl" refers to a branched or unbranched hydrocarbon fragment containing the specified number of carbon atoms and having one point of attachment. Examples include *n*-propyl (a C_3alkyl), *iso*-propyl (also a C_3alkyl), and *t*-butyl (a C_4alkyl).

"Alkylene" refers to a divalent radical which is a branched or unbranched hydrocarbon fragment containing the specified number of carbon atoms, and having two points of attachment. An example is propylene [$-CH_2CH_2CH_2$-, a C_3alkylene].

"Alkylcarboxy" refers to a branched or unbranched hydrocarbon fragment terminated by a carboxylic acid group [-COOH]. Examples include carboxymethyl [$HOOC-CH_2$-, a C_2alkylcarboxy] and carboxyethyl [$HOOC-CH_2CH_2$-, a C_3alkylcarboxy].

"Aryl" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl (also known as heteroaryl groups) and biaryl groups. Carbocyclic aryl groups are generally preferred in the compounds of the present invention, where phenyl and naphthyl groups are preferred carbocyclic aryl groups.

"Aralkyl" refers to an alkylene group wherein one of the points of attachment is to an aryl group. An example of an aralkyl group is the benzyl group [$C_6H_5CH_2$-, a C_7aralkyl group].

"Cycloalkyl" refers to a ring, which may be saturated or unsaturated and monocyclic, bicyclic, or tricyclic formed entirely from carbon atoms. An example of a cycloalkyl group is the cyclopentenyl group (C_5H_7-), which is a five carbon (C_5) unsaturated cycloalkyl group.

"Carbocyclic" refers to a ring which may be either an aryl ring or a cycloalkyl ring, both as defined above.

"Carbocyclic aryl" refers to aromatic groups wherein the atoms which form the aromatic ring are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups such as phenyl, and bicyclic carbocyclic aryl groups such as naphthyl, all of which may be optionally substituted.

"Heteroatom" refers to a non-carbon atom, where boron, nitrogen, oxygen, sulfur and phosphorus are preferred heteroatoms, with nitrogen, oxygen and sulfur being particularly preferred heteroatoms in the compounds of the present invention.

"Heteroaryl" refers to aryl groups having from 1 to 9 carbon atoms and the remainder of the atoms are heteroatoms, and includes those heterocyclic systems described in "Handbook of Chemistry and Physics," 49th edition, 1968, R.C. Weast, editor; The Chemical Rubber Co., Cleveland, OH. See particularly Section C, Rules for Naming Organic Compounds, B. Fundamental Heterocyclic Systems. Suitable heteroaryls include furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, imidazolyl, and the like.

"Hydroxyalkyl" refers to a branched or unbranched hydrocarbon fragment substituted with an hydroxy (-OH) group. Examples include hydroxymethyl (-CH_2OH, a C_1hydroxyalkyl) and 1-hydroxyethyl (-$CHOHCH_3$, a C_2hydroxyalkyl).

"Thioalkyl" refers to a sulfur atom substituted by an alkyl group, for example thiomethyl (CH_3S-, a C_1thioalkyl).

"Patient" refers to a warm-blooded animal such as a mammal which can and will benefit from the above treatment (curative or prophylactic). It is understood that guinea pigs, dogs, cats, rats, mice, horses, cattle, sheep, and humans are examples of male and female patients within the scope of the meaning of the term.

"Pharmaceutically acceptable carriers" for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remingtons Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be used. Id.

"Pharmaceutically acceptable salt" refers to salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts). The compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

The "therapeutically effective amount" or the "therapeutically effective dose" of a compound of the present invention will depend on the route of administration, the type of warm-blooded animal being treated, and the physical characteristics of the specific warm-blooded animal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet,

concurrent medication and other factors which those skilled in the medical arts will recognize.

Compositions described herein as "containing a compound of formula (I)" etc. encompass compositions that contain more than one compound of formula (I), etc.

Compositions described herein as "containing a pro-erectile compound" or the like encompass compositions that contain more than one such compound.

Compounds of the Present Invention

In one aspect, the compounds of the present invention can occupy the following seretonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

In another aspect, the compounds of the present invention can occupy the following seretonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, and 5-HT_3.

In another aspect, the compounds of the present invention can occupy the following seretonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

In another aspect, the compounds of the present invention can provide agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

In another aspect, the compounds of the present invention can provide agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

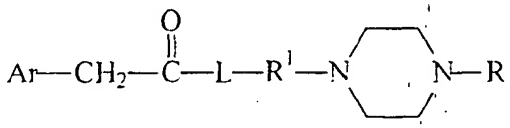
In another aspect, the compounds of the present invention can provide agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

In another aspect, the compounds of the present invention can provide agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

In another aspect, the compounds of the present invention do not interact with the alpha-adrenoceptors.

In another aspect, the compounds of the present invention do not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

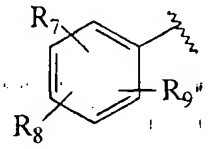
These compounds are referred to herein as "compounds of the invention". Formula (I), as defined below, represents an exemplary group of compounds of the present invention.



(I)

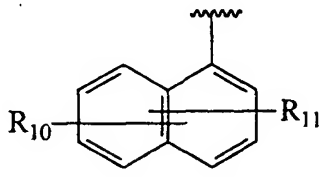
including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):



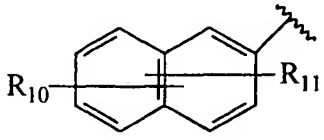
(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



(III)

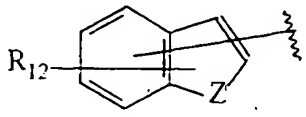
and



(IV)

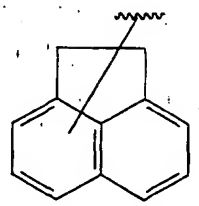
where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl,

C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



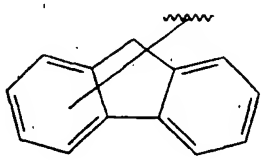
(V)

where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-$CH_2C(O)$-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



(VI)

and



(VII);

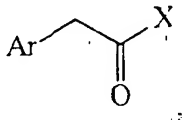
L is selected from the group of a direct bond, O, NH, and $N(C_1$-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

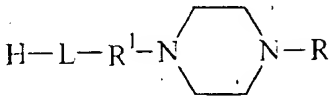
R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

1-Methyl-4-(2-naphthaleneacetyl)piperazine (Compound 1), 1-methyl-4-(1-naphthaleneacetyl)piperazine (Compound 2), 2-(naphthylacetyl)piperazine, (Compound 7) and 1-(naphthylacetyl)piperazine (Compound 8), are some examples of this exemplary group.

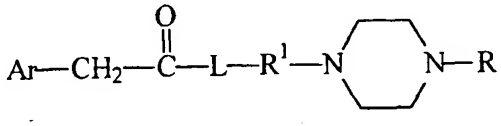
Certain compounds of the invention may be prepared by a method wherein a substituted acetic acid compound or activated version thereof, having the formula



wherein X is OH or an activated (leaving) group such as chloride, is reacted accordingly with a compound of the formula



For compounds where R is H, it is preferred that selective protection of these N-H functions (*e.g.*, in the form of a t-Boc (N-tert-butoxy-carbonyl) group) is carried out prior to reaction with a substituted acetic acid compound or activated version thereof. Examples 3 and 4 illustrate the use of the t-Boc protective group in two different conjugation reactions. Conditions for removal of the t-Boc function are described also. Other suitable protecting groups and conditions for deprotection are set forth in, for example, Greene, "Protective Groups in Organic Chemistry", John Wiley & Son, New York, N.Y. (1991). The reaction provides a bond between C=O and L as shown in the formula below, when the acid chloride contains the Ar group.



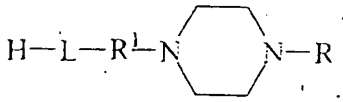
(I).

Compounds of formula Ar-CH_2-C(=O)-X, wherein X is other than -OH, may be prepared from the corresponding acid (where X is -OH). These acid starting materials, such as 1-naphthalene acetic acid, 2-naphthalene acetic acid, phenylacetic acid, bromophenylacetic acid (including the 2-, 3- and 4- positional isomers), methylphenylacetic acid (also known as tolylacetic acid) and many other

compounds of the formula Ar-CH_2-COOH are commercially available. *See, e.g.*, Aldrich Chemical Co., Milwaukee, WI.

A substituted acetic acid may be reacted with, *e.g.*, thionyl chloride, to prepare an activated substituted acetic acid compound. Other synthetic protocols for preparing an activated acid may be found in, *e.g.*, Szmuszkovicz, J.; Von Voigtlander, P.F. (1982) *J. Med. Chem.* 25: 1125-1126; U.S. Patent 5,506,257 to MacLeod B.A. et al., U.S. Patent 5,637,583 to MacLeod B.A. et al. and Clark, C.R. et al. (1988) *J. Med. Chem.* 31: 831-836.

The activated substituted acetic acid compound is then reacted with an amine or alcohol compound (depending on the identity of L) of the formula



For compounds where R^1 is a 1,2-disubstituted C_5-C_6cycloalkyl the following references provide some methods for their preparation. The preparation of 1,2-diaminocyclohexyl intermediates is described in, *e.g.*, Szmuszkovicz, J.; Von Voigtlander, P.F. (1982) *J. Med. Chem.* 25: 1125-1126; and U.S. Patent 5,506,257 to MacLeod B. A. et al. The preparation of 1-hydroxy-2-aminocyclohexyl intermediate is described in U.S. Patent 5,637,583, also to MacLeod B.A. et al. The preparation of reactive carboxylic acid derivatives is described in the above references as well as in Clark, C. R. et al. (1988) *J. Med. Chem.* 31: 831-836.

Alternatively the carboxylic acids may be coupled to the amine in the presence of a coupling reagent such as dicyclohexyl carbodiimide (DCC) or the like. The reaction is generally carried out in a suitable solvent such as tetrahydrofuran or dioxane at ambient temperature, but depending upon the reactivity of the specific starting materials employed, the reaction time, solvent employed and reaction temperature may be varied without undue experimentation by one of ordinary skill in the art, to achieve the desired coupling reaction. A reaction temperature of between about -25°C and the boiling point of the solvent are typically employed. The reaction between the activated carboxylic acid (*e.g.*, acid chloride) and the amine is generally carried out at ambient temperature in a suitable solvent such as chloroform or

dichloromethane in the presence of an acid acceptor (*i.e.*, base) such as a tertiary amine or an alkaline metal carbonate or bicarbonate. The mixture of amine and acid halide is allowed to react until the reaction is essentially complete.

Without intending to be bound, the following is offered as one interpretation of various disclosures found in the literature regarding serotonin receptors.

Serotonin receptors and ligands

Molecular biological and biochemical techniques have confirmed the existence of at least 7 distinct 5-HT receptor types containing an unprecedented total of 15 subtypes (Saudou & Hen, 1995, Adv. Pharmacol. 327; Peroutka, 1994, Neurochem. Int. 533). Four of these receptors, 5-HT_{1-4}, have been characterized pharmacologically (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). The 5-HT_{5-7} receptors, identified through cDNA clones and comparisons of primary sequences, have not been characterized pharmacologically as no selective ligands have been identified; the functional role of these receptors have yet to be explored (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). All of the 5-HT receptors, except the 5-HT_3 subtype (ion channel), belong to a superfamily of G-protein coupled receptors possessing seven transmembrane spanning sequences (Saudou & Hen, 1995, Adv. Pharmacol. 327; Peroutka & Howell, 1994, Neuropharmacol. 319). The following description of 5-HT receptors and ligands will be restricted to those that have been characterized pharmacologically (Chart 1).

Chart 1.

BIOCHEMISTRY AND PHARMACOLOGY OF SOME 5-HT RECEPTORS

Receptor Type	Subtype	G-protein	Second Messenger	Agonist	Antagonist
5-HT_1	A	G_i/G_o	↓ cAMP	8-OHDPAT	WAY100635
	B	G_i/G_o	↓ cAMP	CP93129	Isamoltane
	D	$G_{s/aq}$	↑ cAMP	GR4611	GR127935
5-HT_2	A	$G_{s/aq}$	↑ IP_3	DOI	MDL100097
	B	$G_{s/aq}$	↑ IP_3	5-MeOT	LY266097
	C	$G_{s/aq}$	↑ IP_3	RO600175	RS102221
5-HT_3	2?	None	Ion Channel	1-PBG	BRL46470A
5-HT_4	?	$G_{s/aq}$	↑ cAMP	SC53116	GR113808

Chart 1. 5-HT receptors: their G-proteins, second messengers, agonists and antagonists

5-HT_1 receptors

5-HT_{1A} receptors are encoded by intronless genes which form primary sequences between 365-422 amino acids in length and are found in a wide variety of central and peripheral tissues (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). The actions of agonists at 5-HT_1 receptors are mediated through a reduction in cellular cAMP by activation of an inhibitory G-protein (G_i/G_o) and reduction in adenylate cyclase activity (de Vivo & Maayani, 1986, J. Pharmacol. Exp. Ther. 248). In some tissues the 5-HT_1 receptor is directly coupled to membrane bound potassium (K^+) channel such that receptor activation leads to increased K^+ conductance and cellular hyperpolarization (Andrade et al., 1986).

5-HT_{1A} receptors are distributed in a number of CNS areas involved in the modulation of erectile responses (Laporte et al., 1991, Eur. J. Pharmacol. 59) with particularly dense distribution in the hippocampus (Marcinkiewicz et al., 1984, Brain Res. 159). These receptors act as somatodendritic autoreceptors on 5-HT neurones, terminal heteroreceptors on non-5-HT neurones and as post-synaptic receptors. Activation of any of these types of 5-HT_{1A} receptors is believed to result in a

reduction in neuronal activity which ultimately leads to a broad range of behavioral responses including hypothermia, hyperphagia and the 5-HT "behavioral syndrome" (de Montigny & Blier, 1992, Clin. Neuropharmacol. 610A). Pharmacologically 5-HT_{1A} receptors are marked by a high affinity for the agonist 8-OHDPAT and the antagonist WAY100635 (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157).

5-HT_{1B} receptors are concentrated in basal ganglia and cortical areas and act as both pre-synaptic autoreceptors at 5-HT nerve terminals (Davidson & Stamford, 1997, Brain Res. 238) and heteroreceptors on cholinergic, glutaminergic and dopaminergic nerve terminals (Galloway et al., 1993, Synapse. 90). Activation of 5-HT_{1B} receptors induces changes in locomotion and temperature (Tricklebank et al., 1986, Neuropharmacol. 877) and it has recently been shown that mice lacking 5-HT_{1B} receptors exhibit increased aggressive behavior (Ramboz et al., 1996, Behav. Brain Res. 305). These receptors are poorly characterized pharmacologically as a number of very potent agonists and antagonists exist but have limited selectivity over other 5-HT_1 receptor subtypes (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). At present CP93129 and isamoltane have the greatest selectivity, as agonist and antagonist respectively, over other 5-HT receptor types (Koe et al., 1992, Neurochem. 1268).

5-HT_{1D} receptors in humans have recently been classified as the species homologue (> 95% primary sequence homology but markedly different pharmacological profile) of the rodent 5-HT_{1B} receptor (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). Unlike 5-$HT_{1A/1B}$ receptors, the 5-HT_{1D} receptor is positively coupled to a stimulatory G-protein ($G_{s/\alpha q}$). 5-HT_{1D} receptors reside in a number of brain regions involved in the expression of erection with high densities in the nucleus accumbens, dorsal raphe, locus coeruleus, hippocampus and cortex (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). Development of agonists and antagonists at this receptor subtype was driven by the perceived utility of such compounds in the treatment of migraine associated with neurogenic inflammation (Koe et al., 1992, Neurochem. 1268). Currently GR46611 and GR127935 are regarded as potent and selective 5-HT_{1D} agonists and antagonists respectively (Starkey & Skingle, 1994, Neuropharmacol. 393).

5-HT_2 receptors

5-HT_2 receptors have been subclassified into three distinct subtypes (2A-2C) based on pharmacological and molecular evidence (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). 5-HT_2 receptors share a large degree of overall homology (> 50 %), and even greater homology within the transmembrane spanning sequences (> 70 %), with positive coupling to $G_s/G_{\alpha q}$ and inositoltriphosphate (IP_3) turnover believed to be a common signal transduction pathway (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157).

To a large extent 5-HT_{2A} receptors are located centrally in the cortex, claustrum and basal ganglia and are believed to mediate the hallucinogenic effects and head shaking behaviors of the ergots and L-5-HTP respectively (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). 5-HT_{2A} antagonists have been employed clinically for use in the treatment of hypertension (Barrett & Vanover, 1993, Psychopharmacol. 1) and are currently being explored for their efficacy in reducing the extrapyramidal side effects associated with treatments for Schizophrenia (Barrett & Vanover., 1993, Psychopharmacol. 1; Tricklebank, 1996, Behav. Brain Res. 15). As the 5-HT_{2A} receptors exhibit a great deal of sequence homology with 5-$HT_{2B/2C}$ receptors development of agonists and antagonists which can discriminate the three subtypes has been limited. LSD and other ergot cogners as well as the aminopropanes DOI and DOM have high affinity and agonist properties at 5-HT_{2A} receptors but also have poor selectivity (Baxter et al., 1995, Trends Pharmacol. Sci. 105). Recently amperozide and MDL100907 have been developed and show remarkable potency and selectivity as antagonists of the 5-HT_{2A} receptor subtype (Baxter et al., 1995, Trends Pharmacol. Sci. 105).

5-HT_{2B} receptors and mRNA have been detected in central structures including the amygdala, septum, hypothalamus and cerebellum (Duxon et al., 1997, Neuropharmacol. 601). The 5-HT_{2B} receptor is also distributed heavily in a wide variety of rat peripheral tissues, especially in the stomach fundus (Baxter et al., 1995, Trends Pharmacol. Sci. 105). 5-HT_{2B} receptors mediate contraction of the stomach fundus but may also relax other vascular smooth muscle in a number of species

through endothelium and NO dependent mechanisms (Baxter et al., 1995, Trends Pharmacol. Sci. 105). 5-methoxytryptamine and α-methyl 5-HT are agonists which exhibit a high potency for 5-HT_{2B} receptors whereas yohimbine and rauwolscine (Baxter et al., 1995, Trends Pharmacol. Sci. 105) and the newly developed compound LY266097 exhibits high potency and selectivity in it's antagonist actions at this receptor subtype (Audia et al., 1996, J. Med. Chem. 2773).

5-HT_{2C} receptors are known to exist in extremely high density in the choroid plexus in a number of species including man (Pazos et al., 1987, Neuroscience. 97; Julius et al., 1988, Science. 558). Lower levels of this receptor subtype are found in cortex, hippocampus, striatum, substantia nigra and spinal cord (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157; Helton et al., 1994, Neuroreport. 2617). At present there appears to be no evidence for the expression of 5-HT_{2C} gene products within peripheral tissues (Helton et al., 1994, Neuroreport. 2617). Interestingly the 5-HT_{2C} receptor is the first G-protein coupled receptor which has been shown to exhibit post-translational modification which results in 7 different splice variants, 4 of which have different pharmacological profiles in rat choroid plexus (Burns et al., 1997, Nature. 303). 5-HT_{2C} receptors have been implicated in the regulation of cerebrospinal fluid production (Fisone et al., 1998, Pathways. Mol. Med. 258), migraine (Fozard & Gray, 1989, Arch. Pharmacol. 135) panic disorder (Lucki, 1992, Neurosci. Biobehav. Rev. 83), hyperthermia (Quested et al, 1996, Psychopharmacol. 305), hypophagia and hypolocomotion (Curzon & Kennett, 1990, Trends Pharmacol. Sci. 181). However the ability to determine an unambiguous role of this receptor subtype in any of these functional responses will have to await the use of recently developed ligands (both agonist and antagonist) which exhibit better selectivity than those already in use. At present a variety of compounds representing diverse structural classes act as non-selective agonists at 5-HT_{2C} receptors. MK212, DOI, m-CPP and RO600175 behave as agonists at 5-HT_{2C} receptors but show little selectivity in all cases except RO600175 (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157; Millan et al., 1997a, Eur. J. Pharmacol. 9). Existing antagonists include ritanserin, mianserin and 1-naphthylpiperazine (Schoeffter & Hoyer, 1989; Hoyer et

al., 1994, Pharmacol. Rev. 46 (2): 157) whereas recently developed antagonists include SB242084 and RS102221 (Kennett et al., 1997, Neuropharmacol. 609; Bonhaus et al., 1997, Neuropharmacol. 621).

5-HT$_3$ receptors

The 5-HT$_3$ receptor is a cation permeable pentameric channel protein which is expressed in low levels in cortex, hippocampus and amygdala and at higher levels in peripheral nerves of the autonomic nervous system (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). At present there is evidence to suggest that this receptor may also exist in different forms as splice variants (Hope et al, 1993, Eur. J. Pharmacol. 187) and that a large degree of species variation exists in this receptors affinity for 5-HT$_3$ ligands (Fozard, 1989, Trends Pharmacol. Sci. 307). Administration of 5-HT$_3$ receptor ligands will produce a wide variety of effects depending on the agonist or antagonist nature of the ligand and the basal tone of 5-HT$_3$ receptor mediated activity (Eglen et al., 1993, J. Pharmacol. Exp. Ther. 535). Interest in this receptor subtype is intense due to the potential utility of 5-HT$_3$ receptor antagonists as antiemetics for cancer chemotherapy, cognitive enhancers, and antipsychotics (Fozard & Kalkman, 1992, Curr. Opin. Neurol. Neurosurg. 496). Antagonists include MDL72222, ondansetron and BRL46470A (Gargiulo et al., 1996, Neuropsychobiology. 189). Agonists at 5-HT$_3$ receptors are much more limited in number and utility. 2-Methyl 5-HT, phenylbiguanide and chlorophenylbiguanide are all very potent in their binding to 5-HT$_3$ sites but usually show only partial agonist activity in functional screens *in vitro* and *in vivo* (Delagrange et al., 1996, Eur. J. Pharmacol. 195).

5-HT$_4$ receptors

5-HT$_4$ receptors, unlike 5-HT$_{1A}$ receptors, are thought to be positively coupled to adenylate cyclase through G_s (Saudou & Hen, 1995, Adv. Pharmacol. 327) and have also been reported to alter neuronal calcium activated K^+ conductance (Andrade & Chaput, 1991, Science. 1261). Potent and selective 5-HT$_4$ ligands have

been employed to map distributions of this receptor subtype to areas including the striatum, globus pallidus, substantia nigra, hippocampus and olfactory tubercle (Grossman et al., 1993, Br. J. Pharmacol. 618; Waeber et al., 1994, Neuropharmacol. 527). Centrally, agonist action at this receptor subtype is associated with reduction in neuronal potassium current and both slow and rapid depolarization (Chaput et al., 1990, Eur. J. Pharmacol. 441), thus resulting in a functional increase in nerve output (Bockaert et al., 1990, Mol. Pharmacol. 408; Consolo et al., 1994, Neuroreport. 1230). Peripherally 5-HT_4 agonists cause contraction of a wide variety of vascular and non-vascular smooth muscles (Hedge & Eglen, 1996, FASEB J. 1398). The most potent and selective agonists at 5-HT_4 receptors include SDZ205557 and RS39604 (Hedge et al, 1995, Br. J. Pharmacol. 1087). Based on the effects of agonists at central and peripheral sites 5-HT_4 antagonists have been developed and explored for their utility in the treatment of irritable bowel syndrome (Bockaert et al., 1990, Mol. Pharmacol. 408) and as antipsychotic agents, cognitive enhancers and anxiolytics (Steward et al., 1996, Br. J. Pharmacol. 55; Ge & Barnes, 1996). Selective and potent antagonists at 5-HT_4 receptors include GR113808 and RS23597190 (Bockaert et al., 1990, Mol. Pharmacol. 408; Waeber et al., 1993, Neuroreport. 1239).

Compositions of the Present Invention

The present invention provides compositions, preferably pharmaceutical compositions, which contain at least one compound of the present invention as set forth above, and at least one pharmaceutically acceptable carrier or diluent, where the compounds of the present invention demonstrate selective seretonin (5HT) receptor activity, including funtioning as either (i) a 5HT2c agonist and (simultaneously) a 5HT2a antagnoist, or (ii) simultaneously as a 5HT2c agonist, a 5HT2a antagonist, and a 5HT1a agonist (weak). These compounds may be formulated into a composition in a form including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof.

The composition may include, for example, water. In a preferred embodiment, the composition is in the form of a tablet, and particularly a fast-release

tablet for oral administration. A fast-release tablet (having a rapid disintegration time) is desired in order to provide the patient with a rapid onset of enhanced sexual performance and/or increased libido and/or relief of sexual dysfunction.

A "fast-release" tablet will have a disintegration time of less than about one hour, preferably less than about 20 minutes, and more preferably less than about two or even one minutes. A suitable fast-release tablet contains 40 mg of a compound of the present invention, 8 mg of silicon dioxide (NF), 4 mg of stearic acid (NF), 212 mg of lactose (NF), 120 mg of microcrystalline cellulose (NF) and 16 mg of croscarmellose sodium (NF). A tablet containing these ingredients may be prepared by finely dividing and then mixing each ingredient together, then compressing the mixture into a tablet form. The tablet has a weight of about 400 mg. Other methods of mixing and tablet formulation will be readily apparent to one of ordinary skill in the art. A tablet prepared by this method will typically have a hardness of 10.7 Kp, an average thickness of about 0.2 inches and an average disintegration time of about 45 minutes.

Disintegrant compounds, such as croscarmellose sodium (NF) (available as Ac-Di-Sol from FMC Corporation), may be used to enhance the dissolution time of a formulation of the present invention. Other disintegrants such as potato starch, Explotab™ sodium starch glycolate, Polyplasdone™ XL crospovidone NF, Starch 1500™ pregelatinized starch NF may be employed in the formulations of the present invention. Each of U.S. Patent Nos. 5,731,339, 5,298,261 and 5,079,018 also describe formulations which demonstrate fast disintegration times, which may be employed to prepare a fast release formulation of the present invention.

Suitable disintegrants and methods for measuring disintegration time of tablets include Gissinger et al. "A Comparative Evaluation of the Properties of some Table Disintegrants" *Drug Development and Industrial Pharmacy* 6(5):511-536 (1980); and *European Pharmacopeia* 1980.

The pharmaceutical compositions of the present invention may be in any form which allows for the composition to be administered to a patient. For example, the composition may be in the form of a solid, liquid or gas (aerosol).

Typical routes of administration include, without limitation, oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrameatal, intraurethral injection or infusion techniques. Pharmaceutical composition of the invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the inventive compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions of the invention, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or digylcerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers

such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

A liquid compositions intended for either parenteral or oral administration should contain an amount of the inventive compound such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of a compound of the invention in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Preferred oral compositions contain between about 4% and about 50% of the inventive compound. Preferred compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 1% by weight of active compound.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of the inventive compound of from about 0.1 to about 10% w/v (weight per unit volume).

The composition may be intended for rectal administration, in the form, *e.g.*, of a suppository which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

The compounds of the invention may be administered through use of insert(s), bead(s), timed-release formulation(s), patch(es) or fast-release formulation(s).

It will be evident to those of ordinary skill in the art that the optimal dosage of the compound(s) of the invention may depend on the weight and physical condition of the patient; on the severity and longevity of the sexual dysfunction (when the goal is to treat sexual dysfunction); on the particular form of the active ingredient, the manner of administration and the composition employed. It is to be understood that use of a compound of the invention in a chemotherapy can involve such a compound being bound to an agent, for example, a monoclonal or polyclonal antibody, a protein or a liposome, which assist the delivery of said compound.

Therefore, the invention relates further to a pharmaceutical or veterinary composition comprising an effective amount of a compound of the invention in association with a carrier.

In a further embodiment, the present invention is directed to the use of a compound of the invention (having a receptor profile as identified above, and which includes physiologically acceptable salts and hydrates), for the manufacture of a medicament for treating, relieving or preventing the effects of sexual dysfunction. Thus, the compound(s) of the invention may be used for the manufacture of a medicament for treating, relieving or preventing the effects of male sexual dysfunction, preferably erectile inadequacy and inhibited male orgasm, especially erectile inadequacy. The the compound(s) of the invention may also be used for the manufacture of a medicament for treating, relieving or preventing the effects of female sexual dysfunction, preferably sexual arousal disorder and inhibited femal orgasm, especially sexual arousal disorder.

In a further embodiment, the present invention provides a method for the treatment of a male or female patient suffering from sexual dysfunction, or a method to prevent sexual dysfunction in a patient (having, for example, a history of sexual dysfunction) comprising the administration thereto of a therapeutically or prophylactically effective amount of a compound(s) of the invention or a composition

including same, as provided above. The sexual dysfunction may be, for example, male erectile dysfunction or impotence. A patient that cannot obtain an erection may be treated according to the present invention, while a patient that cannot maintain an erection may receive a prophylactic dose of a compound of the invention in order to prevent premature loss of an erection.

In a still further embodiment, the present invention provides a method for increasing the libido of a male or female patient comprising the administration thereto of a therapeutically effective amount of a compound(s) of the invention or a composition including same, as provided above.

In a still further embodiment, the present invention provides a method for enhancing the sexual performance of a male or female patient that is not necessarily exhibiting symptoms of sexual dysfunction, comprising administering to the patient in need thereof a therapeutically or prophylactically effective amount of a compound of the invention, or a composition including same, as provided above. Enhanced sexual performance occurs when there is an increase in the type of behavior that is typically associated with the patient's sexual activity or interest in sexual activity. Increased tone in the patient's genitals is one indication of an enhancement of sexual performance. Enhancement of sexual performance may result in, *e.g.*, a pro-erectile response in the patient, or an improvement in erectile function such as any increase in the ability of the patient maintain an erection, induce or improve ejaculation (e.g., have multiple ejaculations within a shortened period of time), or induce or improve orgasm. Specific examples of enhancements in sexual performance are described in connection with the pharmacological testing of compounds and compositions of the present invention, as set forth herein.

The term "therapeutically effective amount" or "therapeutically effective dose" refers to an amount which is effective, upon single or multiple dose administration to the patient, to enhance the libido and/or sexual performance of the patient receiving the compound or a composition containing the compound as provided above. Such an amount may serve to treat a sexual dysfunction, *e.g.*, impotence in males, and/or to enhance the sexual desire and/or sexual performance of

a patient without a sexual dysfunction. For example, the therapeutically effective amount may be administered to, for example, a bull, to promote increased semen ejaculation, where the ejaculated semen is collected and stored for use as it is needed to impregnate female cows in promotion of a breeding program. Increased sexual ejaculation is an example of enhanced sexual performance according to the present invention.

A therapeutically or prophylactically effective amount of a substituted acetic acid derivative of the invention is expected to vary from about 0.01 milligram per kilogram of body weight per day (mg/kg/day) to about 200 mg/kg/day. Preferred amounts are expected to vary from about 0.5 to about 80 mg/kg/day. A pharmaceutical composition containing a compound(s) of the invention may contain between 0.01 and 1% by weight of the active ingredient, and between about 5 and 10% by weight glucose in order to increase the osmolarity of the solution. Two illustrative compositions are (1) 5 mg/mL of a compound(s) of the invention and distilled water in 100 mL total volume, and (2) 5 mg/mL of a compound(s) of the invention, 25 mg/mL glucose, and distilled water in 100 mL total volume.

In effecting treatment of a patient in need of an agent for treating sexual dysfunction and/or enhancing sexual performance and/or a pro-libido agent, a compound of the invention can be administered in any form or mode which makes the compound bioavailable in effective amounts, including oral, aerosol, and parenteral routes. For example, compounds of the invention can be administered orally, by aerosolization, subcutaneously, intramuscularly, intravenously, transdermally, intranasally, rectally, topically, and the like. The compounds of the invention may be administered by direct injection into, *e.g.*, the corpus cavernosa (intracavernously). The compounds of the invention may be administered intraurethrally (*e.g.*, via an intraurethral catheter). The compounds of the invention may be administered topically, *e.g.*, directly to the penis. The compounds may be administered intrameatally. Oral or aerosol administration is generally preferred. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the compound selected,

the condition to be treated, the stage of the condition, and other relevant circumstances. *See, e.g.*, Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (1990).

The compounds can be administered alone or in the form of a pharmaceutical composition in combination with pharmaceutically acceptable carriers or excipients, the proportion and nature of which are determined by the solubility and chemical properties of the compound selected, the chosen route of administration, and standard pharmaceutical practice.

In another embodiment, the present invention provides compositions comprising a compound(s) of the invention in admixture or otherwise in association with one or more inert carriers. These compositions are useful, for example, as assay standards, as convenient means of making bulk shipments, or as pharmaceutical compositions. An assayable amount of a compound of the invention is an amount which is readily measurable by standard assay procedures and techniques as are well known and appreciated by those skilled in the art. Assayable amounts of a compound of the invention will generally vary from about 0.001% to about 75% of the composition by weight. Inert carriers can be any material which does not degrade or otherwise covalently react with a compound of the invention. Examples of suitable inert carriers are water; aqueous buffers, such as those which are generally useful in High Performance Liquid Chromatography (HPLC) analysis; organic solvents, such as acetonitrile, ethyl acetate, hexane and the like; and pharmaceutically acceptable carriers or excipients.

More particularly, the present invention provides pharmaceutical compositions comprising a therapeutically effective amount of a compound(s) of the invention, in admixture or otherwise in association with one or more pharmaceutically acceptable carriers or excipients.

The pharmaceutical compositions are prepared in a manner well known in the pharmaceutical art. The carrier or excipient may be a solid, semi-solid, or liquid material which can serve as a vehicle or medium for the active ingredient. Suitable carriers or excipients are well known in the art. The pharmaceutical composition may

be adapted for oral, parenteral, or topical use and may be administered to the patient in the form of tablets, capsules, solution, suspensions, or the like.

The compounds of the present invention may be administered orally, for example, with an inert diluent or with an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like. These preparations should preferably contain at least 4% of the compound of the invention as an active ingredient, but this amount may be varied depending upon the particular form and may conveniently be between 4% to about 70% of the weight of the unit. The amount of the compound present in compositions is such that a suitable dosage will be obtained. The tablets, pills, capsules and the like may also contain one or more of the following adjuvants: binders such as microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch or lactose, disintegrating agents such as alginic acid, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; and sweetening agents such as sucrose or saccharin may be added or a flavoring agent such as peppermint, methyl salicylate or orange flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or a fatty oil. Other dosage unit forms may contain other various materials which modify the physical form of the dosage unit, for example, as coatings. Thus, tablets or pills may be coated with sugar, shellac, or other enteric coating agents. A syrup may contain, in addition to the present compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

For the purpose of parenteral therapeutic administration, the compounds of the present invention may be incorporated into a solution or suspension. These preparations should contain at least 0.1% of a compound of the invention, but this amount may be varied to be between 0.1 and about 50% of the

weight thereof. The amount of the inventive compound present in such compositions is such that a suitable dosage will be obtained. Preferred compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 1% by weight of active compound.

The compounds of the present invention may also be administered by aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system which dispenses the active ingredients. Aerosols of compounds of the invention may be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the active ingredient. Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, spacers and the like, which together may form a kit. Preferred aerosols are able to be determined by one skilled in the art.

The compounds of this invention may also be administered topically, and when done so the carrier may suitably comprise a solution, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Topical formulations may contain a concentration of the inventive compound of from about 0.1 to about 10% w/v (weight per unit volume).

The solutions or suspensions may also include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred carrier or diluent.

The compound(s) of the invention may be combined with one or more known pharmacological agents used in the treatment and/or prevention of sexual dysfunction and/or known to enhance the libido and/or sexual performance of a patient receiving the pharmacological agents.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

In the following Examples, unless otherwise indicated, the reactants, reagents and solvents were of standard commercial grade, and were obtained from Aldrich Chemical Co., Milwaukee, WI, or a similar chemical supply house

EXAMPLE 1

PREPARATION OF 1-METHYL-4-(1-NAPHTHALENEACETYL)PIPERAZINE MONOHYDROCHLORIDE (COMPOUND 2.HCL)

Acid Chloride formation: 1-naphthylacetic acid (5.00 g, 26.8 mmol) was refluxed in thionyl chloride (10mL) under nitrogen for 1 hour. The mixture was stirred at room temperature for a further 1 hour, and the thionyl chloride was removed *in vacuo* (using 1x10 mL, 2x5 mL CCl_4) to leave an oil which was dissolved in dichloromethane (50 mL).

Amide formation: The acid chloride solution was added *via* cannula to a cooled (-78°C) solution of 1-methylpiperazine (2.69 g, 26.8 mmol) in dichloromethane (50 mL) under nitrogen. The resulting thick white suspension was filtered and washed with ether (3x10mL) and dried to provide a first crop (3.06 g). A second crop (1.05 g) was collected from the filtrate.

Microanalysis: C 66.30, H 6.96, N 9.13% (theoretical for $C_{17}H_{21}N_2OCl$: C 66.99, H 6.94, N 9.19%).

EXAMPLE 2

PREPARATION OF 1-METHYL-4-(2-NAPHTHALENEACETYL)PIPERAZINE MONOHYDROCHLORIDE (COMPOUND 1.HCL)

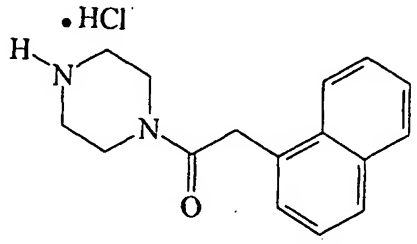
Acid chloride formation: 2-naphthylacetic acid (3.90 g, 21.0 mmol) was refluxed in thionyl chloride (10mL) under nitrogen for 1 hour. The mixture was stirred at room temperature for a further 1.5 hour before the thionyl chloride was removed *in vacuo* (using 1x10 mL, 2x5 mL CCl_4). The residue, an orange solid, was dissolved in dichloromethane (7 mL).

Amide formation: The acid chloride solution was added *via* cannula to a cooled (ice bath) solution of 1-methylpiperazine (2.0 g, 20 mmol) in dichloromethane (10 mL) under nitrogen. Additional dichloromethane (25 mL) was added in order to reduce the viscosity of the reaction medium since a great deal of white solid precipitated from solution almost immediately. The mixture was stirred at room temperature for 30min. The crude product (5.44 g) was filtered off and washed with ether (3x15 mL). The product was pumped on the vacuum line then dissolved in hot methanol. Further cooling (slowly) provided a solid precipitate (3.19 g). The product was filtered, washed with ether and dried *in vacuo*.

Microanalysis: C 66.60, H 7.27, N 9.12% (theoretical for $C_{17}H_{21}N_2OCl(0.5\ H_20)$: C 65.06, H 7.07, N 8.93%).

EXAMPLE 3

1-(NAPHTHYLACETYL)PIPERAZINE.MONOHYDROCHLORIDE (COMPOUND 8.HCL)



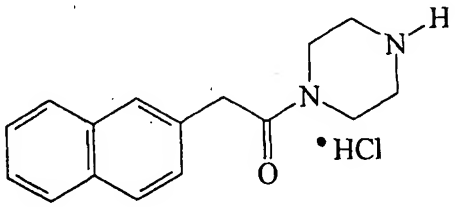
To a mixture of 1-naphthylacetic acid (1.07 g, 5.7 mmol), *tert*-butyl 1-piperazine carboxylate (1.0 g, 5.4 mmol) and triethylamine (0.7 mL) in anhydrous

tetrahydrofuran (50 mL) was added portionwise benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate "Py-BOP" (2.97 g, 5.7 mmol). The reaction mixture was then stirred at room temperature for 5 hours. The precipitate was filtered off and the solvent was evaporated *in vacuo*. The residue was taken up with water (30 mL) and the aqueous solution was basified by addition of 5M NaOH aqueous solution (10 mL). The basic aqueous solution was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were dried over sodium sulfate and the solvent was evaporated *in vacuo* to yield the intermediate N-protected piperazine suitable for the next step without any further purification.

The N-protected piperazine was treated with 3M HCl ethyl acetate solution (5 mL) to provide a precipitate which was recrystallized from a mixture of toluene-methanol-diethyl ether (1:1, v/v, 30 mL) to yield the title compound. NMR analyses (proton and C-13) and mass spectroscopic analysis of the product are consistent with the structure indicated.

EXAMPLE 4

2-(NAPHTHYLACETYL)PIPERAZINE.MONOHYDROCHLORIDE (COMPOUND 7.HCL)



2-Naphthylacetic acid (1.86 g, 10 mmol) was refluxed in thionyl chloride (10 mL) for 1 h. After stirring at room temperature for a further 1.5 h, the thionyl chloride was removed *in vacuo* (using 1 x 10 mL and 2 x 5 mL CCl_4). The residue was dissolved in dichloromethane (15 mL). Then the acid chloride solution was added via cannula to a cooled solution (ice bath) of *tert*-butyl 1-piperazine carboxylate (1.86 g, 10 mmol) and triethylamine (1.4 mL, 10 mmol) in dichloromethane (15 mL) under nitrogen. The reaction mixture was diluted with dichloromethane (60 mL) and washed with 1M HCl aqueous solution (50 mL), water

(30 mL), 1M sodium bicarbonate aqueous solution (50 mL) and water (30 mL). The organic layer was collected, dried over sodium sulfate and concentrated *in vacuo* to yield the crude intermediate carbamate suitable for the next step without any further purification.

The above carbamate dissolved in ethyl acetate (100 mL) was treated with HCl saturated ethyl acetate solution (50 mL). After a few minutes a precipitate was formed and the reaction mixture was stirred for another 4 hours in order to complete the reaction. The yellow precipitate was collected and recrystallized from ethanol to yield 1.98 g of the title compound. NMR analyses (proton and C-13) and mass spectroscopic analysis of the product are consistent with the structure indicated.

PHARMACOLOGICAL TESTING

EXAMPLE 5

BINDING AFFINITIES OF COMPOUNDS OF INVENTION FOR SUBTYPES OF SEROTONIN RECEPTORS (5-HT)

Methods

The binding affinities of compounds of the present invention for the different subtypes of serotonin receptors (5-HT) can be performed using with some modifications, standard methods reported in the literature.

The materials and procedures used for each 5-HT subtype receptor assay are briefly described below.

5-HT_{1A}: (Hoyer et al. 1985, Eur. J. Pharmacol. 13; Schoeffter et al. 1989, Naunyn-Schmiedberg Arch. Pharmacol. 135). Receptor Source: Human recombinant expressed in HeLa cells. Radioligand: [^{3}H]-8-OH-DPAT (100 Ci/mmol); Final ligand concentration – [0.25 nM]. Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgCl, 0.5 mM EDTA, and 0.1% Ascorbic acid at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined

and compared to control values in order to ascertain any interactions of test compound with the cloned 5-HT_{1A} binding site.

5-HT_{1B}: (Hoyer et al., 1985; Eur. J. Pharmacol. 13; Schoeffter et al. 1989, Naunyn-Schmiedberg Arch. Pharmacol. 135). Receptor Source: Rat striatal membranes. Radioligand: [^{125}I]-Iodocyanopindolol (2200 Ci/mmol); Final ligand concentration – [0.15 nM]. Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.4) containing 60 µM (-) isoproterenol at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5-HT_{1B} binding site.

5-HT_{1D}: (Waeber et al., 1988, Naunyn-Schmiedberg Arch. Pharmacol. 595). Receptor Source: Bovine striatal membranes Radioligand: [3H]-5-Carboxamidotryptamine (5-CT) (20-70 Ci/mmol). Final ligand concentration – [0.75 nM].

Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 4mM $CaCl_2$, 100 nM 8-OH-DPAT, 100 nM Mesulergine, 10 µM pargyline and 0.1% ascorbic acid at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5-HT_{1D} binding site.

5-HT_{2A}: (Leysen et al., 1982, Mol. Pharmacol. 301; Martin et al., 1994, Neuropharmacol. 261). Receptor Source: Rat cortical membranes. Radioligand: [3H] Ketanserin (60-90 Ci/mmol). Final ligand concentration – [1.0nM]. Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.6) at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5-HT_2 binding site.

5-HT_{2C}: (Pazos et al., 1985b, Eur. J. Pharmacol. 539; Hoyer et al., 1985, Eur. J. Pharmacol. 13). Receptor Source: Pig choroid plexus membranes.

Radioligand: [3H] Mesulgerine (50-60 Ci/mmol). Final ligand concentration – [1.0 nM]. Incubation Conditions: Reactions are carried out in 50 mM TRIS-HCl (pH 7.7) containing 4mM $CaCl_2$ and 0.1% ascorbic acid at 37°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5-HT_{2C} binding site.

5-HT_3: (Lummis et al., 1990. Eur. J. Pharmacol. 223; Hoyer et al., 1988. Mol. Pharmacol. 303; Tyers. 1991. Therapie. 431). Receptor Source: N1E-115 cells. Radioligand: [3H]-GR65630 (30-70 Ci/mmol). Final ligand concentration – [0.35nM]. Incubation Conditions: Reactions are carried out in 20 mM HEPES (pH 7.4) containing 150 mM NaCl at 25°C for 60 minutes. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the 5-HT_3 binding site.

Results

The binding affinity results for two of the compounds of the present invention (Compound 1 and Compound 7) for six subtypes of serotonin receptors (5-HT) are shown in Table 1. Results are reported as % inhibition of binding of specific radioligands to their respective 5-HT subtype receptors by the test compounds.

Table 1

AFFINITIES OF COMPOUNDS OF INVENTION FOR SUBTYPES OF SEROTONIN RECEPTORS (5-HT)

% INHIBITION OF RADIOLIGAND BINDING TO SUBTYPES OF 5-HT RECEPTOR

	5-HT Receptor					
Test Compound	1A	1B	1D	2A	2C	3
Compound 1	NS	NS	NS	72%	51%	60%
Compound 7	NS	NS	NS		39%	83%

Table 1 shows the effects of Compound 1 (10^{-5}M) and Compound 7 (10^{-5}M) on the binding of specific radioligands to their respective 5-HT subtype receptors as indicated below.

[3H]-8-OH-DPAT/5-HT_{1A}; [125I]-Iodocyanopindol/5-HT_{1B}; [3H]-5-Carboxamidotryptamine (5-CT)/5-HT_{1D}; [3H]-Ketanserin/5-HT_{2A}; [3H]-Mesulergine/5-HT_{2C}; [3H]-GR65630/5-HT_3. Data are expressed as the % inhibition of radioligand binding by the test compound at the concentrations used. NS denotes results are less than 20% and are generally considered to be non-significant for these assays.

EXAMPLE 6

THE EFFECTS OF COMPOUNDS OF THE PRESENT INVENTION AND OTHER RELATED COMPOUNDS ON ERECTILE RESPONSES OF MALE RATS.

Methods

Male Sprague-Dawley (SD) rats (n=10 per test group) were housed in groups of six, or in pairs, under ambient temperature and light or under reversed 12 hour light cycle and air condition temperature (approx. 22 ° C) respectively with standard rat chow and water available ad libitum. Drugs were tested for their ability to induce erection in male rats according to methods described by Berendsen et al. (1990, Br. J. Pharmacol. 667). Briefly, animals were injected via an intraperitoneal (i.p.) route with saline or test drug and placed individually in clear Plexiglas® cages of 45 x 25 x 25 cm for behavioral observation. Erection was recorded for test animals exhibiting an abrupt upright posturing motion with repeated licking of the engorged penis. Rats housed in groups of six were treated with saline or Compound 1 (2.0-64.0 mg/kg) and, 5 minutes later, observed for the appearance of erection and the latency to first erection over 90 minutes. Rats housed in pairs were treated with saline, Compound 1 (2.0-32.0 mg/kg), Compound 4 (0.1-10.0 mg/kg), Compound 3 (2.0-32.0 mg/kg), Compound 5 (0.1-10.0 mg/kg), Compound 6 (0.1-10.0 mg/kg), Compound 2 (2.0-32.0 mg/kg), m-CPP (0.2-3.12 mg/kg), TFMPP (0.2-3.12 mg/kg) or 1-NP (0.2-

3.12 mg/kg) 5 minutes prior to 60 minutes observation for the occurrence of erection. As rats were used more than once in the second series of experiments at least 72 hours washout was given between drug tests. Dose-response data was analysed by ANOVA followed by Dunnett's test for multiple comparisons.

Results

Compound 1 enhanced erection during 90 minutes of observation in a dose-dependent manner. A clearly defined maximum of 7.7 ± 0.4 erections was elicited at a dose of 16 mg/kg. Saline treated rats exhibited 1.2 ± 0.4 erections over 90 minutes of observation (Figure 1). The time to the appearance of the first erection was reduced in a dose-dependent manner. A minimal latency to first erection of 654 ± 108 seconds also occurred at a dose of 16 mg/kg (Figure 1). At the highest dose tested the number of erections was significantly increased as compared to saline treated animals whereas the time to first erection was significantly reduced compared to saline controls. All animals (100%) treated with Compound 1 exhibited erection whereas only 60% of saline treated animals exhibited erection (Figure 1).

In rats observed for penile erection over 60 minutes, compounds of the present invention such as Compound 1 and Compound 2 elicited erection in a dose-dependent manner with greater maximal efficacy, but less potency, than the halogenated arylpiperazines m-CPP and TFMPP. Control animals exhibited 0.5 ± 0.1 and 0.7 ± 0.1 erections in 60 minutes of observation in tests with Compound 1and Compound 2, and m-CPP-1-NP respectively. Compound 1 and Compound 2 were maximally effective at a dose of 16.0 mg/kg eliciting 3.7 ± 0.4 and 3.2 ± 0.6 erections respectively ($p < 0.01$ vs. saline; Figure 2).

Diagram 1

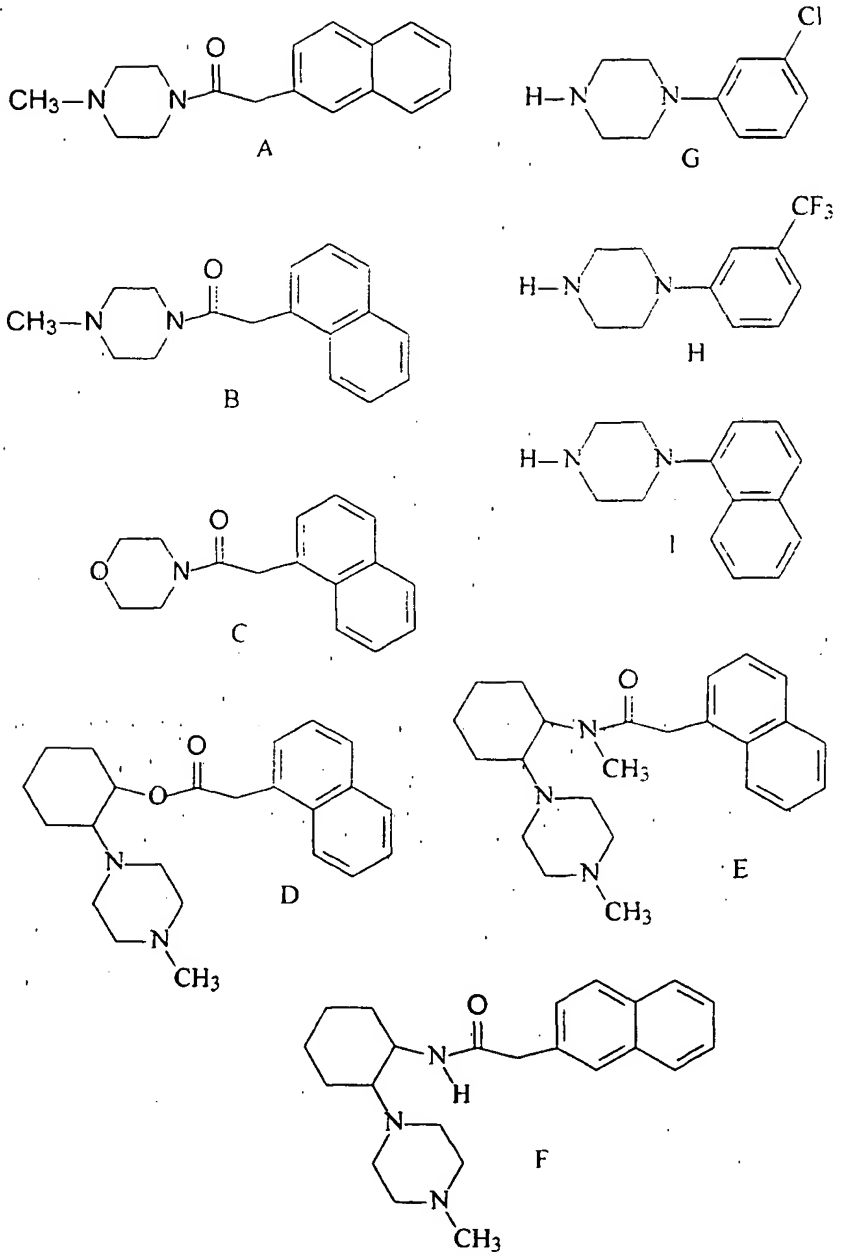


Diagram 1. Structural formulae of some of the compounds tested in this study. A, Compound 1; B, Compound 2; C, Compound 3; D, Compound 4; E, Compound 5; F, Compound 6; G, meta-chlorophenylpiperazine (m-CPP); H, trifluoromethyl-phenylpiperazine (TFMPP); I, 1-Naphthylpiperazine (1-NP).

m-CPP and TFMPP elicited maximal effects of 2.6 ± 0.2 and 1.8 ± 0.3 erections at doses of 0.75 and 1.0 mg/kg respectively ($p < 0.01$ vs. saline; Figure 3).

The erection enhancing action of Compound 1 is dose-related with significant increases in erection at all of the doses tested (2.0-32.0 mg/kg). The erectogenic actions of Compound 2 were significant at all but the lowest dose tested (2.0 mg/kg). Other related compounds showed varying degrees of pro-erectile activity. The non-piperazine analog, Compound 3, failed to increase the appearance of erection, as compared to saline treated rats, at any of the doses tested (2.0-32.0 mg/kg) ($p > 0.15$ vs. saline; Figure 2). 1-NP, a non-substituted 1-naphthyl arylpiperazine, failed to elicit erection at any of the doses tested but did significantly reduce erection at the highest dose tested (10.0 mg/kg) ($p < 0.5$ vs. saline; Figure 3).

Compounds in which a cyclohexane bridge was placed between the piperazine moiety and the amide nitrogen or ester oxygen (see D-F in Diagram 1) had less consistent and significant effects on the appearance of erection. Rats treated with saline exhibited 0.9 ± 0.1 erections in 60 minutes of observation. Compound 4 (cyclohexylester) increased erection significantly to 1.9 ± 0.4 erections at a dose of 0.1 mg/kg but failed to increase erection significantly at higher doses (0.1-10.0 mg/kg) (Figure 4). Compound 6 on the other hand increased the appearance of erection to 2.0 ± 0.3 erections but only at a dose of 10.0 mg/kg (Figure 4). Compound 5 was without significant effect on spontaneous erectile responses at any of the doses tested (0.1-10.0 mg/kg) ($p > 0.15$ vs. saline; Figure 4).

A number of similar experiments were carried out to demonstrate the pro-erectile property of some other compounds of the invention. Results of these experiments are briefly described below.

At a dosage of 8 mg/kg, Compound 8 showed an average of 4.5 erections per rat (n=3) over a 30 minutes time period of observation with a 100% response rate, whereas in the control rats (n=3, saline) an average of 1.5 erections per rat was observed over the same duration with a 67% response rate.

In another set of experiments, at dosage of 3 mg/kg, Compound 7 showed an average of 3.25 erections per rat (n=4) over a 30 minutes time period of observation with a 100% response rate; and at dosage of 10 mg/kg, Compound 7 showed an average of 4.8 erections per rat (n=4) over a 30 minutes time period of

observation with a 100% response rate. Whereas in the control rats (n=4, saline) an average of 1.75 erections per rat was observed over the same duration with a 100% response rate. Latency to the first erection for the treated animals was also decreased to an average value of 290 seconds (3 mg/kg) and 336 seconds (10 mg/kg) from the control average value of 492 seconds. In the same set of experiments, Compound 1 at dosage of 10 mg/kg showed an average of 4.8 erections per rat (n=4) over a 30 minutes time period of observation with a 100% response rate. The average latency to the first erection was 252 seconds.

EXAMPLE 7

THE EFFECTS OF COMPOUNDS OF THE PRESENT INVENTION ON ERECTILE AND DESIRE RESPONSES IN MALE PRIMATES.

Methods

i) **Dose-response study**. Male Macaca *fascicularis* (n=6) were housed individually under ambient light and temperature conditions with access to a fruit supplemented chow diet and water ad libitum. Test animals were taken from the housing facility and transferred, at a site remote from the housing facility, to an observational cage of the same dimensions as the housing cage. Twenty minutes after cage transfer animals were injected with either saline (0.9%) or Compound 1 (1.0-10.0 mg/kg) in a volume of 0.5 ml/kg (i.p.). Animals were then immediately transferred to an empty observational room where, after 10 minutes, penile responses, pursed-lip gesturing and yawning were scored for 1 hour according to methods described by Pomerantz (1991, Pharmacol. Biochem. Behav. 123). Briefly, every 10 seconds the animal was scored for penile tumescence and arousal of the following intensity: grade 0, penile region visible but glans penis not visible; grade 1, glans penis clearly visible; grade 2, penis extended but not fully erect; grade 3, erect penis (less than 90 ° angle between penis and animals trunk); grade 4, erection with masturbation and grade 5, erection with masturbation and ejaculation. Pursed-lip gesturing and yawning, affiliative signals believed to be part of courtship behavior (Pomerantz, 1990,

Pharmacol. Biochem. Behav. 659) were counted as the number of such behaviors in 1 hour of observation.

ii) **Single dose study**. In a second set of experiments adult male Macaca *fascicularis* (3.8-8 kg; n=6) were injected with saline, Compound 1, and other related compounds of the present invention (1.0 mg/kg; i.p.; 0.5 ml/kg body weight) and observed for behavioral responses and locomotion for 1 hour following injection. Monkeys used for testing were separated from the cluster of housing cages and, dosed and observed in pairs that had visual contact with one another, in the middle of the housing environment.

In the Compound 1 dose-response study, test doses were randomized and a rotation was established such that each monkey received each dose with at least a three day interval between doses. Data for the dose-response study was analysed by repeated measures ANOVA with Dunnett's test for multiple comparisons. In the single dose study a rotation was established such that of the paired monkeys one received saline and the other received test drug. Therefore data for each compound is limited to n=3 whereas the saline treated group numbers 18. Due to the limited numbers of drug treated animals obtained in this study statistical analysis was not performed on proportional data and comparisons are qualitative.

Results

i) **Dose response study**. In male primates observed in isolation, Compound 1 produced a dose-dependent increase in grade 1 penile erections (Table 2). There was a dose-related trend for an increase in the number of grade 2 and grade 3 responses (Table 2). Compound 1 produced a dose-related trend for increase in pursed-lip gesturing which reached significance at a dose of 10.0 mg/kg (Table 3).

Table 2

EFFECTS OF COMPOUND 1 ON MALE PRIMATE ERECTILE RESPONSES

ISOLATED OBSERVATION DOSE-RESPONSE STUDY

	Erectile Response					
Treatment	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
(mg/kg)	% Genital Observations					
Compound 1 (0.1)	81.8 ± 8.2	17.8 ± 7.9	0.4 ± 0.3	0 ± 0	0 ± 0	0 ± 0
Compound 1 (0.3)	67.3 ± 12.2	32.8 ± 11.5	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Compound 1 (1.0)	72.0 ± 12.0	26.6 ± 10.6	1.4 ± 1.4	0 ± 0	0 ± 0	0 ± 0
Compound 1 (3.0)	56.0 ± 7.8	42.1 ± 8.3	1.7 ± 1.4	0.3 ± 0.3	0 ± 0	0 ± 0
Compound 1 (10.0)	24.5 ± 7.6	72.1 ± 7.7*	2.5 ± 1.0	0.9 ± 0.6	0 ± 0	0 ± 0
Saline (0.9%)	68.7 ± 11.8	31.3 ± 11.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 2 shows the effects of Compound 1 on erectile responses in male primates observed in isolation. Data are expressed as mean ± SEM for 6 test animals. Penile responses were scored every 10 seconds for 1 hour according to the following scale: grade 0, penile region visible but glans penis not visible; grade 1, glans penis clearly visible; grade 2, penis extended but not fully erect; grade 3, erect penis (less than 90 ° angle between penis and animals trunk); grade 4, erection with masturbation and grade 5, erection with masturbation and ejaculation. An asterisk indicates significant difference from saline as determined by repeated measures ANOVA and Dunnett's test for multiple comparisons ($p < 0.05$).

Table 3

EFFECTS OF COMPOUND 1 ON MALE PRIMATE LIBIDO RESPONSES

ISOLATED OBSERVATION STUDY

	Affiliative Behaviors	
Treatment (mg/kg)	Pursed-Lip Gesture	Yawn
Compound 1 (0.1)	5.8 ± 3.9	8.8 ± 4.5
Compound 1 (0.3)	10.2 ± 3.9	11.7 ± 3.8
Compound 1 (1.0)	7.5 ± 3.4	11.3 ± 2.5
Compound 1 (3.0)	14.0 ± 8.3	13 ± 3.4
Compound 1 (10.0)	33 ± 14*	14.0 ± 4.0
Saline	6.5 ± 4.5	8.2 ± 2.7

Table 3 shows the effects of Compound 1 on affiliative behaviors in male primates observed in isolation. Data are expressed as mean ± SEM for 6 test animals. Pursed-lip gestures and yawning are described as the absolute number of events during 60 minutes of observation. An asterisk indicates significant difference from saline as determined by repeated measures ANOVA and Dunnett's test for multiple comparisons ($p < 0.05$).

ii) **Single dose study.** In primates observed in a paired setting within the housing environment, compounds of the present invention in general increased the number of animals having full erection (grade 3) as well as increased the number of libido responses (Table 4).

Table 4

EFFECTS ON PENILE & BEHAVIORAL RESPONSES IN MALE PRIMATES

PAIRED OBSERVATION SINGLE DOSE STUDY

	Compound						
	CPD 1	CPD 2	CPD 3	CPD 4	CPD 5	CPD 6	Saline
Penile/Libido Response	% Responders						
	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=3)	(n=18)
Grade 0	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Grade 1	100 %	100 %	100 %	100 %	67 %	100 %	100 %
Grade 2	100 %	67 %	67 %	100 %	67 %	67 %	22 %
Grade 3	67 %	33 %	0 %	100 %	67 %	33 %	6 %
Grade 4	33 %	33 %	0 %	100 %	67 %	0 %	6 %
Grade 5	0%	0 %	0 %	33%	0 %	0 %	6 %
	Number of Libido Responses						
Yawn	14	8	4	14	2	7	6
Pursed-Lip Gesture	10	9	1	6	5	10	1

Table 4 shows the effects of Compound 1 and other related compounds on penile and affiliative behavior responses in male primates observed in a paired setting. Data are expressed as the percentage of test animals having a particular grade of penile response. Penile responses were scored every 10 seconds for 1 hour according to the following scale: grade 0, penile region visible but glans penis not visible; grade 1, glans penis clearly visible; grade 2, penis extended but not fully erect; grade 3, erect penis (less than 90 ° angle between penis and animals trunk); grade 4, erection with masturbation and grade 5, erection with masturbation and ejaculation. Affiliative behavioral responses were scored as the absolute number of events during 60 minutes of observation.

EXAMPLE 8

5-HT_{2C} AND 5-HT_{1A} RECEPTOR DEPENDENCE OF THE COMPOUND 1 ERECTION ENHANCING ACTIONS

Methods

The involvement of 5-HT_{2C} and 5-HT_{1A} receptors in the erection enhancing actions of Compound 1 was elucidated using the chronic administration of the 5-HT_{2C} antagonists ritanserin and mianserin and the acute administration of the 5-HT_{1A} antagonist NAN-190. The 5-HT_{2C} receptor interaction study was also to determine whether or not functional pre-synaptic serotonergic nerve terminals are required for the expression of the Compound 1 erectile responses.

i) **5-HT_{2C} receptor interaction**. Male SD rats (n=5 per test group) housed in groups of 5 were injected i.p (1.0 ml/kg body weight) daily with saline, vehicle, low (0.3 mg/kg) or high (3.0 mg/kg) doses of mianserin or ritanserin for 10 days. Each drug had its own saline and vehicle control groups. Rats were injected i.p. with saline or a maximally effective erectogenic dose of Compound 1 (16 mg/kg), 24 hours after the last dose of ritanserin and 48 hours after the last dose of mianserin respectively. Rats were observed for 30 minutes for the appearance of erection as described previously (Example 6). Animal body weights were recorded for each of the treatment groups on days 0, 1, 3, 5, 7 and 9. Weight gain was assessed at 7 days as the difference from pre-drug weights whereas absolute weight was recorded on day 9.

To determine whether the Compound 1 response was mediated by actions on pre- versus post-synaptic sites, the 5-HT synthesis inhibitor (pCPA) was used to destroy pre-synaptic serotonergic inputs. pCPA (150 mg/kg) or saline (0.9%; 1.0 ml/kg) was administered i.p. to male SD rats (n=8 per test group) at 24 hour intervals for 3 days. Twenty-four hours after the last dose of pCPA or saline the animals were injected i.p. with Compound 1 (16 mg/kg) and observed for the appearance of erection for 30 minutes as described previously in Example 6.

ii) **5-HT_{1A} receptor interaction**. Male SD rats (n=5 per test group) housed in groups of 5 were injected i.p (1.0 ml/kg body weight) with saline or NAN-190 (0.02-2.0 mg/kg) 30 minutes prior to i.p. injection with a maximally effective erectogenic dose of Compound 1 (16 mg/kg). Observations of erectile responses were carried out for 30 minutes as described previously (Example 6).

All data was analyzed by ANOVA with Dunnett's test for multiple comparisons. $P < 0.05$ was taken as a significant result in all cases.

Results

Previous work with 5-HT_2 ligands has shown that 5-HT_{2A} and 5-HT_{2C} receptors respond differently to acute versus chronic treatment with agonists and antagonists (Aulakh et al., 1994, J. Pharmacol. Expl Ther. 127; Mazzola-Pomietto et al., 1996). Most notably, hyperthermia induced by the 5-$HT_{2A/2C}$ agonist DOI and the 5-HT_{2C} agonist m-CPP were significantly reduced by mianserin and ritanserin administered acutely. Chronic administration of mianserin specifically inhibited the DOI induced hyperthermia but not m-CPP induced hyperthermia with the reverse scenario being true for chronic ritanserin administration (Mazzola-Pomietto et al., 1997, Psychopharmacol. 144). These results, and others, suggest that the hyperthermia induced by DOI and m-CPP are mediated by 5-HT_{2A} and 5-HT_{2C} receptors respectively and that, chronic mianserin treatment preferentially induces functional desensitization of 5-HT_{2A} receptors whereas chronic ritanserin preferentially desensitizes 5-HT_{2C} receptors (Sanders-Bush et al., 1987, Eur. J. Pharmacol. 199; Smith et al., 1990J. Pharmacol. Exp. Ther. 484; Barker et al., 1994, J. Biol. Chem. 11687; Westphal & Sanders-Bush, 1994Mol. Pharmacol. 937; Hartman & Northup, 1996,J. Biol. Chem. 22591).

For sometime the 5-HT_{2C} receptor, formerly known as the 5-HT_{1C} receptor (Humphrey et al., 1993, Trends Pharmacol. Sci. 233), has been implicated in the erection enhancing actions of apomorphine (Protais et al., 1995, Psychopharmacol. 376), oxytocin (Stancampiano et al., 1994, Eur. J. Pharmacol. 149) and the non-selective 5-HT agonists MK212, m-CPP and TFMPP (Bagdy et al., 1992, Eur. J.

Pharmacol. 9; Stancampiano et al., 1994, Eur. J. Pharmacol. 149; Berendsen et al., 1996, Eur. J. Pharmacol. 308). These conclusions were based on experiments which, due to the paucity of even remotely selective ligands for this receptor subtype, involved exclusion of other possible 5-HT receptor subtypes. Therefore antagonism of erectile responses with ritanserin or mianserin, but not ketanserin, has often been taken as a reasonably good indication that the response involves 5-HT_{2C} receptors. Recently certain physiological characteristics of 5-HT_{2C} receptor activation and antagonism have been elucidated which also help to confirm the role of 5-HT_{2C} receptors in a given drugs actions (Newton et al., 1996).

i) **5-HT_{2C} interaction.** A high dose of ritanserin (3.0 mg/kg) administered daily for 10 days significantly reduced the erection enhancing actions of Compound 1 to a level which was not significantly different from saline treated animals (Figure 5; $p > 0.05$). Erectile responses in the presence of a high dose of ritanserin were significantly less than erectile responses in vehicle treated animals. Although the vehicle used for dissolution of ritanserin was 100% DMSO this treatment also failed to affect the test animals responses to Compound 1 despite causing noticeable discomfort on injection. The low dose (0.3 mg/kg) of ritanserin, although producing a slight reduction, was without significant effect on the Compound 1 induced erectile responses.

Mianserin failed to attenuate the erection enhancing actions of Compound 1 at any of the doses tested. However there was an insignificant reduction in the number of Compound 1 induced erections at a low dose (0.3 mg/kg) which was not evident at the higher dose of 3.0 mg/kg (Figure 6).

Daily administration of pCPA for 3 days failed to affect erection elicited by a test dose of Compound 1 (16 mg/kg). Compound 1 elicited 3.3 ± 0.8 erections in rats pre-treated with saline whereas test animals pre-treated with pCPA showed erectile responses of 3.9 ± 1.0 erections after i.p. administration of Compound 1. Saline and pCPA treated rats receiving Compound 1 had significantly higher erectile responses as compared to animals receiving only saline which exhibited

erectile responses of 0.8 ± 0.3 erections ($p < 0.05$ versus test groups receiving Compound 1).

The differential results obtained for mianserin and ritanserin on the erection enhancing actions of Compound 1 indicate that the 5-HT_2 receptor mediating the erection enhancing actions of Compound 1 is the 5-HT_{2C} subtype. Berendsen et al. (1990, Br. J. Pharmacol. 667) have shown that DOI, a 5-$HT_{2A/2C}$ agonist which, on its own, does not induce erection, produces a dose-dependent increase in erection when combined with the 5-HT_{2A} antagonists pirenperone and spiperone. Under the same conditions DOI induced headshakes were dose-dependently antagonized by the 5-HT_{2A} antagonists thus providing very good evidence for a 5-HT_{2C} mediated erectile response and a 5-HT_{2A} mediated headshake response. Berendsen and Broekkamp (1991, Psychopharmacol. 219) have also shown that chronic administration of mianserin fails to attenuate the erection enhancing actions of the 5-HT_{2C} agonist MK212 and that chronic administration of mianserin unmasks erection enhancing actions of DOI, while at the same time inhibiting the DOI headshake response. Taken together with published observations, the ability of ritanserin, but not mianserin, to significantly reduce the erection enhancing actions of Compound 1 in the present study strongly implicates that the mechanism mediating these actions of the compounds of the present invention is critically dependent upon functional 5-HT_{2C} receptors.

Results obtained with the 5-HT synthesis inhibitor pCPA provide support for a direct action on post-synaptic 5-HT_{2C} receptors. An indirect action mediated by release of 5-HT maybe excluded on the grounds that 1) erections produced by the 5-HT releaser fenfluramine are significantly reduced in animals pre-treated with pCPA (Maeda et al., 1994b, Brain Res. 181;) and 2) pCPA fails to inhibit the erection enhancing actions of compounds having documented affinity and activity at 5-HT_{2C} receptors (Protais et al., 1995, Psychopharmacol. 376). Although brain levels of 5-HT and its metabolite 5-HIAA were not measured in this study there is an abundance of evidence to support that selective and significant reduction of these two compounds in several brain areas occurs at the doses used in this study (Blackshear et

al., 1981, Eur. J. Pharmacol. 325; Quik & Azmita, 1983, Eur. J. Pharmacol. 377; Protais et al., 1995, Psychopharmacol. 376). The slight increase in Compound J induced erectile responses in pCPA pre-treated rats is in agreement with other studies which showed a slight functional 5-HT_{2C} receptor supersensitivity in response to pCPA administration (Conn & Sanders-Bush, 1986, J. Neuroscience. 3669; Protais et al., 1995, Psychopharmacol. ; Curzon et al., 1997, Trends Pharmacol. Sci. 21).

ii) **5-HT_{1A} receptor interaction**. From the available literature, the role of 5-HT_{1A} receptors in erection is not well understood. While it is clear that 5-HT_{1A} receptor agonists (full) inhibit erection mediated by a number of different mechanisms the same simplicity is not apparent for the actions of 5-HT_{1A} antagonists on erectile responses. Simon et al. (1993, Neuroreport. 229) have demonstrated that tertatolol, a β-adrenergic receptor antagonist having high affinity antagonist properties at 5-HT_{1A} receptors (Prisco et al., 1993), enhances erection induced by the 5-HT re-uptake inhibitors fenfluramine and fluoxetine and the 5-HT_{2C} agonist m-CPP. In the same study, labetolol, a β-adrenergic receptor antagonist having very low affinity for 5-HT_{1A} receptors, but also having significant α-adrenergic receptor antagonist actions. inhibited erection produced by fluoxetine and m-CPP but not fenfluramine. Protais et al. (1995) have demonstrated that tertatolol does not enhance m-CPP induced erection directly but does reverse the inhibitory actions of the strong 5-HT_{1A} agonists 8-OHDPAT and S14506.

Despite the inconsistent effects of tertatolol on the m-CPP induced erection responses in the aforementioned studies the effects of this compound can be ascribed to prevention of an inhibitory interaction of 5-HT_{1A} receptors with the erection enhancing actions of 5-HT_{2C} receptors. The nature of the 5-HT_{1A} receptor mediated inhibition of 5-HT_{2C} receptor induced erections is transmission dependent (i.e. tertatolol does not increase erection on its own (Simon et al., 1993, Neuroreport. 229) and involves post-synaptic receptors (*i.e.*, pCPA pre-treatment does not diminish the inhibitory action of 8-OHDPAT on m-CPP induced erection (Protais et al., 1995, Psychopharmacol. 376).

Compound 1 at a maximally effective erectogenic dose (16/mg/kg) increased erection from 0.6 ± 0.2 erections in control rats to 2.8 ± 0.8 erections (Figure 7; $p < 0.05$ versus control rats). Ascending doses of NAN-190 inhibited the erection enhancing actions of Compound 1. At the lowest dose of NAN-190 tested (0.02 mg/kg) Compound 1 was able to elicit erection that was significantly greater than that observed in control rats. Compound 1 induced erections were reduced to a level not different from control animals at doses of 0.2 and 2.0 mg/kg NAN-190 with erectile responses of 1.0 ± 0.0 erections and 1.0 ± 0.3 erections respectively. In Example 8 it was shown that pindolol. a compound having β-adrenergic and 5-HT_{1A} antagonist action (Sanchez et al., 1996, Eur. J. Pharmacol. 245), inhibited the erection enhancing actions of Compound 1. To clarify the role of 5-HT_{1A} receptors in the Compound 1 responses. NAN-190, a compound having both 5-HT_{1A} and α-adrenergic antagonist actions (Gobert et al., 1995. J. Pharmacol. Exp. Ther. 1032) was studied. NAN-190 dose-dependently inhibited the erection enhancing actions of Compound 1, which is in agreement with the results obtained with pindolol. Support for a mechanism involving differential actions at 5-HT_{1A} receptors comes from the observation that NAN-190 does not act as an antagonist at β-adrenergic receptors (Millan et al., 1995, , J. Pharmacol. Exp. Therap. 1418) but does diminish the erection enhancing actions of Compound 1. As mentioned previously NAN-190 is an effective α_1 receptor antagonist and it is therefore possible that it is these properties which produce its inhibitory actions on the Compound 1 induced erection responses. In summary, the above examples support the findings that the erection enhancing actions of Compound 1 are induced by activation of 5-HT_{2C} receptors at post-synaptic sites within the CNS.

EXAMPLE 9

THE EFFECTS OF COMPOUND 1, M-CPP AND TFMPP ON ERECTILE AND EJACULATORY RESPONSES OF MALE RATS.

From a procreative perspective pharmacological enhancement of erection loses its value as a treatment for erectile dysfunction if semen production

and/or ejaculation are adversely affected. The benefits of pharmacotherapy for the induction of ejaculation are obvious with respect to spinalized patients (Hultling et al., 1995, Hum. Reprod. 847) and veterinary medicine (Chung et al., 1996, Obstet. Gynecol. 22) but, the utility of such an action in normal subjects is debatable (Gorzalka et al., 1990, Ann. NY. Axad. Sci. 435). In preliminary cardiovascular studies it was found that intravenous administration of Compound 1 was effective in inducing seminal emission in addition to pro-erectile activity. However it was not certain whether this effect was a result of the experimental setting or drug treatment or a combination of both. Therefore the following experiments were performed to compare and contrast the effects of maximally effective erection enhancing doses of Compound 1, m-CPP and TFMPP on seminal emission in male rats.

a. Methods

Male SD rats housed in groups of four under reversed light dark cycle were injected i.p. with saline (1.0 ml/kg; n=15), Compound 1 (16.0 mg/kg; n=15), m-CPP (0.75 mg/kg; n=15) or TFMPP (1.0 mg/kg; n=15) 5 minutes prior to observations of paired rats, in a Plexiglas chamber (40 x 40 x 20 cm), for 1 hour for the occurrence of erection, ejaculation, grooming (penile and non-penile) and yawning. Erections were scored as describe previously (section B1.) and ejaculation was scored by visual inspection of the rats eating the ejaculate plug. Grooming was scored as being either penile or non-penile according to Sachs et al. (1988, *The physiology of reproduction.* 1393); with penile grooming being restricted to the genital region and non-penile grooming being any grooming bouts at any region other than the genitalia. Movement was scored as the number of rears during 1 hour and the total number of quadrant crosses during 6 two minute observation periods at 10 minute intervals. Rectal temperature was recorded by thermometer before, and 60 minutes after, saline or drug administration.

Results

At a dose of 16 mg/kg Compound 1 significantly increased erection and ejaculation from 0.8 ± 0.3 erections and 0.3 ± 0.1 ejaculations in saline treated animals to 5.1 ± 0.6 erections and 1.5 ± 0.3 ejaculations (Figure 8). m-CPP (0.75 mg/kg) and TFMPP (1.0 mg/kg) induced 2.0 ± 0.2 and 1.5 ± 0.3 erections respectively, far fewer erections than Compound 1; and failed to induce ejaculation in any of the animals tested (Figure 8).

Locomotion assessed as the number of rearing events was not significantly affected by any of the test drugs. Saline treated animals reared 85 ± 6 times and exhibited 28 ± 4 crosses compared to Compound 1 treated animals which reared 76 ± 5 times and crossed 24 ± 3 times during 60 minutes of observation (Figure 9). m-CPP and TFMPP treated animals displayed rearing behavior of 80 ± 7 and 100 ± 10 rears respectively which were not significantly different from saline treated controls. However, locomotion in terms of crossing events were significantly reduced to 9 ± 2 and 12 ± 2 crosses in m-CPP and TFMPP treated animals respectively (Figure 9).

Additionally, Compound 1 increased grooming of the genital region from 7.5 ± 0.8 bouts in saline treated animals to 12.5 ± 1.7 bouts. m-CPP and TFMPP both reduced genital grooming to 4.8 ± 1.0 and 2.6 ± 0.5 bouts respectively (Figure 10). Non-genital grooming was also significantly reduced by m-CPP and TFMPP to 8.0 ± 1.0 and 7.3 ± 1.2 bouts respectively from a value of 20.7 ± 2.0 bouts in saline controls. Compound 1 failed to significantly alter non-genital grooming bouts with 21.5 ± 3.0 bouts recorded (Figure 10).

In earlier Examples it was shown that Compound 1 was more efficacious but less potent than m-CPP and TFMPP in the elicitation of erection despite having a similar dose-response profile (i.e. a clearly defined maximal effect) and, that Compound 1 enhanced erection via a 5-HT_{2C} post-synaptic receptor dependent mechanism. In the present study the three drugs had markedly different effects on seminal emission. Compound 1 induced seminal emission in 15/15 test animals whereas m-CPP and TFMPP failed to induce emission in any of the 15 test

animals. The type of profile exhibited by Compound 1 as an example of the compounds of the present invention has not been previously reported in the literature for other erectogenic compounds.

Based on comparison to available literature concerning seminal emission in rats, it is likely that the effects of Compound 1 on emission involve interactions with 5-HT neurotransmission. The possibility for direct interactions with the adrenergic system which is predominant in seminal emission responses (Sachs & Meisel, 1988, *The physiology of reproduction.* 1393) appears to be unlikely based on *in vitro* observations with Compound 1.

EXAMPLE 10

THE EFFECTS OF COMPOUND 1 ON COPULATORY PERFORMANCE IN MALE RATS.

In Example 9, Compound 1 was shown to posses markedly different, and stimulatory actions on seminal emission compared to the erectogenic compounds m-CPP and TFMPP in freely moving rats. These actions may be of benefit, from a procreative perspective, as a potential pharmacotherapy for erectile dysfunction. From a prosexual perspective arousal and performance are important aspects of sexual behavior (Schiavi & Segraves, 1995, Psychiatr. Clin. North Am. 7). Previous work has demonstrated that m-CPP and TFMPP reduce copulatory performance in male rats chiefly as a result of an increase in ejaculatory threshold through 1) a reduction in the incidence of ejaculation, 2) an increase in ejaculation latency and 3) an increase in the number of mounts preceding ejaculation (Fernandez-Guasti et al., 1989, Pharmacol. Biochem. Behav. 811Pharmacol. Biochem. Behav. 811; Mendelson & Gorzalka, 1990; Fernandez-Guasti & Rodriguez-Manzo, 1992, Pharmacol. Biochem. Behav. 529; Fernandez-Guasti et al., 1992, Eur. J. Pharmacol. 121). Unlike m-CPP and TFMPP, the selective 5-HT_{1A} agonist 8-OHDPAT improves copulatory performance in rats through reduction in ejaculatory threshold (Glaser et al., 1991, *Brain 5HT1A receptors: behavioral and neurochemical pharmacology.* 106; Johanssen et al., 1991, Eur. J. Pharmacol. 81) and, is thought to enhance arousal through a reduction in latency to mount, intromission and resumption of copulation after ejaculation

(Ahlenius & Larsson, 1991b, *Brain 5-HTA1 receptors: behavioral and neurochemical pharmacology*. 185). However, unlike m-CPP and TFMPP, 8-OHDPAT is known to inhibit erectile responses in most species studied to date (Schnur et al., 1989, Physiol. Behav. 897; Pomerantz et al., 1993a,Eur. J. Pharmacol. 227). To gain a better understanding of the potential for adverse effects of Compound 1 on other aspects of sexual behavior, a rat model of copulatory performance was used to determine its effects on appetitive and consumptive patterns of sexual behavior.

Methods

Male and female Long-Evans rats (500-650 g) housed under 12 hour reverse light cycle were used in this study. Male rats were injected with saline or Compound 1 (1.5-15.0 mg/kg) 20 minutes prior to being paired with an ovariectomized and primed (10 μg estradiol benzoate and 500μg progesterone at 48 hours and 4 hours prior to experiment respectively) female (Tanco et al., 1993, Experientia. 238). For 30 minutes after drug administration latency to first mount, intromission, ejaculation and the number of mounts and intromissions preceding ejaculation were measured. In addition the total number of ejaculations per 30 minute session were recorded. The time after the first ejaculation to the start of the next copulatory sequence (post-ejaculatory interval) was also determined (Watson & Gorzalka, 1992,Neurosci. Lett. 25).

Results

At a maximally effective erectogenic dose, Compound 1 (15 mg/kg) failed to significantly alter mount latency or intromission latency despite a trend for longer intromission latencies in drug treated animals (Table 5). The number of mounts preceding ejaculation were not significantly affected by Compound 1 pre-treatment despite a dose-related trend for a reduction in this variable ($p = 0.15$). However there was a significant and dose-related trend for reduction in the number of intromissions preceding ejaculation (Table 5). In addition Compound 1 caused a significant and dose-related increase in the number of ejaculations during 30 minutes

of observation. Ejaculation latency was not affected in a dose-related manner but was moderately reduced to a level approaching the established level of significance at a dose of 15 mg/kg ($p = 0.05$). Compound 1 failed to significantly alter the post-ejaculatory interval at any of the doses tested despite a dose related trend for reduction in mean differences compared to controls.

Table 5

EFFECTS OF COMPOUND 1 ON COPULATORY PERFORMANCE IN MALE RATS

	Treatment (mg/kg)					
Copulation Variable	Saline (0)	Compound 1 (1.5)	Saline (0)	Compound 1 (7.5)	Saline (0)	Compound 1 (15.0)
Mount Latency (sec)	153 ± 17	218 ± 24	191 ± 22	227 ± 35	157 ± 21	204 ± 32
Intromission Latency (sec)	254 ± 29	294 ± 26	232 ± 25	307 ± 28	247 ± 38	405 ± 88
Ejaculation Latency (sec)	612 ± 33	573 ± 39	486 ± 36	571 ± 42	416 ± 28	331 ± 32
Mounts (#)	11.8 ± 0.8	10.4 ± 0.7	13.5 ± 0.9	8.9 ± 1.0	10.9 ± 2.6	6.6 ± 1.0
Intromissions (#)	9.3 ± 0.5	9.9 ± 0.4	10.7 ± 0.5	7.9 ± 0.4*	9.1 ± 0.5	6.9 ± 0.7*
Ejaculations (#)	1.7 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	4.2 ± 0.1	4.7 ± 0.2*
Post Ejaculatory Interval (sec)	361 ± 38	403 ± 28	383 ± 30	417 ± 39	426 ± 29	447 ± 28

Table 5 shows the effects of Compound 1 (1.5-15.0 mg/kg) on copulatory behaviour in male rats. Data are expressed as mean ± SEM for 15 animals at each dose with a 15 animal saline treated control group at each dose. Latency values for mount, intromission, ejaculation and post-ejaculatory interval are in seconds whereas mounts intromissions and ejaculations are number per 30 minutes observation. An asterisk indicates significant difference compared to saline control ($p < 0.05$; t-test).

It was found that Compound 1 enhanced copulatory performance by reducing the number of intromissions preceding ejaculation and by increasing the number of ejaculations during 30 minutes of observation. This profile is distinctly

different from the copulatory behavior profiles of the erectogenic piperazine 5-HT agonists m-CPP and TFMPP. Both of the latter compounds have been shown to reduce the proportion of animals exhibiting mounting behavior and increase the number of mounts and intromissions preceding ejaculation (Fernandez-Guasti et al., 1989, Pharmacol. Biochem. Behav. 811; Mendelson & Gorzalka, 1990). This action is particularly marked for TFMPP, which, at maximally effective erectogenic doses (1.0 mg/kg), completely suppresses copulatory behavior (Fernandez-Guasti et al., 1989, Pharmacol. Biochem. Behav. 811). m-CPP on the other hand has a less marked effect on mounts and intromissions but does reduce the percentage of animals achieving ejaculation significantly at maximally effective erectogenic doses (Fernandez-Guasti et al., 1989, Pharmacol. Biochem. Behav. 811). When injected directly into the nucleus accumbens or the pre-optic area both 5-HT and TFMPP increase the number of mounts and intromissions preceding ejaculation and ejaculation latency (Fernandez-Guasti et al., 1992). Systemic administration of the 5-HT_{1B} agonist RU24969 produces inhibitory effects on copulation which parallel those produced by systemic administration of m-CPP and TFMPP (Fernandez-Guasti et al., 1989, Pharmacol. Biochem. Behav. 811). In contrast to the actions of 5-HT_{1B} agonists the selective 5-HT_{1A} agonist 8-OHDPAT reduces mounts and intromissions preceding ejaculation and reduces ejaculation latency (Fernandez-Guasti & Rodriguez-Manzo, 1992; Fernández-Guasti et al., 1992).

Results obtained above suggest that in a rat model of copulatory performance Compound 1 has an activity profile which more closely resembles that of a 5-HT_{2A} antagonist and/or a 5-HT_{1A} agonist. From binding studies and functional biochemical assays it is known that 8-OHDPAT displays a 2000 fold selectivity ratio for 5-HT_{1A} versus 5-HT_{1B} receptors (Schoeffter & Hoyer, 1989). m-CPP and TFMPP on the other hand exhibit 5-HT_{1A}/5-HT_{1B} selectivity ratios of 2.5 and 1.3 only. By analogy these results suggest that the inhibitory action of m-CPP and TFMPP are due to activity at 5-HT_{1B} receptors and in this model, Compound 1 was found to lack any significant 5-HT_{1B} agonist activity. This assumption is in line with results from the previous Example where it was demonstrated that Compound 1 enhanced seminal

emission whereas m-CPP and TFMPP prevented seminal emission at doses which produced other 5-HT_{1B} mediated behaviors.

As mentioned previously the full and partial 5-HT_{1A} agonists 8-OHDPAT and ipsapirone facilitate copulatory performance in a manner which is largely determined by a reduction in the number of mounts and intromissions preceding ejaculation. The effects of these drugs on variables believed to reflect arousal state (mount and intromission latency and post-ejaculatory interval) have been reported but were equivocal (Ahlenius & Larson, 1991b, Brain 5-HT1A receptors: behavioral and neurochemical pharmacology. 185; Glaser et al., 1991, Brain 5-HT1A receptors: behavioral and neurochemical pharmacology. 106; Johanssen et al., 1991, Eur. J. Pharmacol. 81). Compound 1 failed to affect variables associated with arousal but did reduce variables associated with performance in a manner similar to those previously described for other 5-HT_{1A} agonists/partial agonists. The only apparent corollary to the interpretation of a potential 5-HT_{1A} mediated enhancement of copulatory performance is the lack of evidence for high affinity binding of Compound 1 to 8-OHDPAT labelled sites in rat cortical membranes (in vitro binding study) and a lack of functional antagonism of apomorphine induced erection. In vitro binding results suggest that direct full activation of 5-HT_{1A} receptors by Compound 1 is unlikely.

In vitro binding assays showed that Compound 1 has strong affinity to ketanserin labelled 5-HT_2 sites in rat cortical membranes. In the previous section it was also suggested that the Compound 1 induced seminal emission could arise from an antagonist action at 5-HT receptors with a reduction in tonic suppression of emission mediated by endogenous 5-HT. Interestingly 5-HT_2 antagonists have also been reported to either have no effect on copulatory performance (Klint et al., 1992, Eur. J. Pharmacol. 241; Klint & Larsson, 1995, Eur. J. Pharmacol. 241), or facilitate it (Ahlenius et al., 1980, Psychopharmacol. 217; Menendez-Abraham et al., 1988, Behav. Brain. Res. 251). However all the experiments mentioned in the above references used 5-HT_2 antagonists with poor 5-HT subtype selectivity. Furthermore, these antagonists were poor in their selectivity at other monoamine receptors known to

adversely affect sexual function, particularly those of the α_1 adrenoceptor subtype (Mendelson & Gorzalka, 1985, Pharmacol. Biochem. Behav. 565; Gorzalka et al., 1990). Using a series of potent and selective 5-HT_2 antagonists based on an ergoline structure lacking α_1 binding capacity, Foreman et al. (1989) have demonstrated a marked drug-induced facilitation of sexual function as measured by a reduction in ejaculation latency. However in these studies drug effects on other sexual response variables were not reported.

In the present study Compound 1 reduced ejaculation latency to the point of significance at a maximally effective erectogenic dose thus implicating the involvement of a 5-HT_2 receptor antagonist action in the copulatory enhancing effects of this compound. It must be noted that any possible 5-HT_2 receptor antagonist action would have to be of either the 5-HT_{2A} or 5-HT_{2B} subtype as the erection enhancing actions of Compound 1 were clearly shown to be mediated by 5-HT_{2C} receptors (Example 8.). Furthermore functional expression of putative 5-$HT_{2A/2B}$ antagonism would have to be co-expressed with 5-HT_{2C} agonist actions as 5-HT_{2A} and 5-HT_{2B} antagonists are not known to elicit erections on their own (Berendsen & Broekkamp, 1987, Eur. J. 279). On the other hand 5-HT_{2A} antagonists have been shown to unmask the erection enhancing actions of mixed 5-$HT_{2A/2C}$ agonists (Berendsen & Broekkamp, 1991, Psychopharmacol. 219).

EXAMPLE 11

THE EFFECTS OF COMPOUND 1 ON COPULATION IN SEXUALLY EXHAUSTED MALE RATS.

In Example 10 it was shown that Compound 1 enhanced copulatory performance as measured by a reduction of mounts and intromissions prior to ejaculation and elevated the number of ejaculations per test series as compared to saline treated animals. However Compound 1 failed to promote sexual response variables indicative of arousal state (mount and intromission latency). It has been proposed that desire or motivational aspects of sexual function may be different than those of arousal and may be assessed through employment of sexually exhausted male rats (Karen & Barfield, 1975, J. Comp. Physiol. Psychol. 693). To better understand

the apparent beneficial effects of Compound 1 on copulatory performance of male rats, Compound 1 was administered to sexually exhausted rats in order to assess its effects on motivational aspects of copulation.

Methods

Male SD rats (500-600 g; n=34) were assessed for their ability to copulate to ejaculation, and resume copulation after ejaculation, on 3 separate occasions at 72 hour intervals. Rats were paired with an estrogen (25 µg, t= -48 hours) and progesterone primed (1.5 mg, t= -4 hours) female (n=18) 5 minutes after being placed in circular wire mesh cages 26 cm in diameter. Animals were excluded from use in further studies if they 1) had an ejaculation latency longer than 30 minutes or 2) a post-ejaculatory interval of longer than 15 minutes (Sachs and Barfield, 1976) on any one of the three pre-drug tests. None of the original cohort had a post-ejaculatory interval longer than 15 minutes such that animals included in the drug study had ejaculation latencies shorter than 30 minutes.

Rats selected for drug study (n=27) were housed individually and allowed to copulate to satiation (4 hours with novel female at 0.5 hour intervals). Twenty-four hours after copulation to satiation the rats were injected with either saline (1.0 ml/kg; n=7), Compound 1 (2.0 mg/kg; (n=7) and 16.0 mg/kg; (n=7)) or 8-OHDPAT (0.2 mg/kg; (n=6)) and were again paired with a receptive female. Behavioral responses were measured as proportions of animals exhibiting mount, intromission, ejaculation and resumption of copulation after the first ejaculation. The latencies to mount, intromission, ejaculation and resumption of copulation after the first ejaculation, and the number of mounts and intromissions preceding ejaculation were recorded.

Results

After screening 34 male rats for ability to copulate to ejaculation, and resume copulation after ejaculation, a total of 27 animals were available for the drug study. Analysis of variance for time effect indicated that none of the variables

measured were significantly different between the three pre-drug testing periods. Therefore cohort mean values (n=81; 27 * 3) were derived for mounts (10.7 ± 0.6), intromissions (22 ± 0.7), mount latency (62.7 ± 3.3 sec), intromission latency (113.3 ± 5.3 sec), ejaculation latency (1033.9 ± 24.9 sec) and post-ejaculatory interval (290.0 ± 7.6 sec) in all animals prior to drug testing.

The proportion of saline treated sexually exhausted animals exhibiting mount (2/7), intromission (2/7) and ejaculation (2/7) was significantly reduced compared to the same animals' responses in pre-drug testing (all 7/7; $p < 0.05$ for all variables; Table 6). However due to the low numbers of saline treated rats resuming copulation after the first ejaculation (1/2) a significant difference could not be detected. Sexually exhausted animals receiving Compound 1 at a dose of 2.0 mg/kg showed a significant reduction in the number of animals exhibiting mounts (3/7), intromission (2/7) and ejaculation (2/7) compared to pre-drug testing (all 7/7; $p < 0.05$ for all variables; Table 6). Of the 2 animals that did ejaculate both also resumed copulation (2/2) such that no significant drug effect was detected for this response variable. In sexually exhausted rats receiving a higher dose of Compound 1 (16.0 mg/kg) the proportion of animals exhibiting mounts (5/7), intromission (5/7), and ejaculation (5/7) were not significantly different from proportions of animals showing similar behaviors in pre-drug testing (7/7; $p > 0.05$ for all variables; Table 6). Compound 1 at a higher dose was unable to significantly effect the proportion of sexually exhausted rats undertaking resumption of copulation after the first ejaculation (3/5). Sexually exhausted rats receiving 8-OHDPAT (0.2 mg/kg) failed to show a significant difference between pre-drug testing and drug testing for the proportion of animals exhibiting mounts (6/6), intromission (6/6), ejaculation (5/6) and resumption of copulation (5/5) ($p > 0.05$ for all variables versus pre-drug proportions of 6/6; Table 6).

Table 6

EFFECTS OF COMPOUND 1 & 8-OHDPAT IN SEXUALLY EXHAUSTED RATS

	Pre-drug	Pre-drug	Sexually Exhausted			
Variable	Sal/Cpd 1	DPAT	Saline (0)	Compound 1 (2.0)	Compound 1 (16.0)	DPAT (0.2)
Mount	7/7	6/6	2/7*	3/7*	5/7+	6/6+
Intromission	7/7	6/6	2/7*	2/7*	5/7+	6/6+
Ejaculation	7/7	6/6	2/7*	2/7*	5/7+	5/6+
Resumption of Copulation	7/7	6/6	1/2	2/2	3/5	5/5+

Table 6 shows the effects of saline (1.0 ml/kg; n=7), Compound 1 (2.0 and 16.0 mg/kg; n=7) and 8-OHDPAT (DPAT; 0.2 mg/kg; n=6) on copulatory performance in sexually exhausted male rats. Data are expressed as the proportion of animals exhibiting mount, intromission, ejaculation and resumption of copulation behaviors. An asterisk indicates significant difference compared to pre-drug control ($p < 0.05$; t-test) whereas a + indicates a significant difference from sexually exhausted animals treated with saline.

As the number of saline treated rats exhibiting various behavioral displays was low after sexual exhaustion the effects of the high dose of Compound 1 and 8-OHDPAT on the number and latency of the measured variables could not be compared to a control sexually exhausted group. Therefore variables measured in sexually exhausted rats treated with Compound 1 (16 mg/kg) and 8-OHDPAT (0.2 mg/kg) were compared to each other. At the highest dose of Compound 1 tested 2 of the test subjects failed to exhibit mounting or intromission within 15 minutes and were therefore excluded from further analysis.

Compound 1 and 8-OHDPAT did not have disparate effects on arousal variables in sexually exhausted rats with mount and intromission latencies of 61 ± 10 seconds and 134 ± 21 seconds and, 65 ± 5 seconds and 99 ± 12 seconds for Compound 1 and 8-OHDPAT respectively ($p > 0.70$ and $p > 0.10$ for mount and intromission latencies respectively). Latency to first ejaculation was significantly shorter at 384 ± 37 seconds in 8-OHDPAT treated sexually exhausted rats compared

to 825 ± 108 seconds in exhausted rats receiving Compound 1. 8-OHDPAT (5/5) was more effective than Compound 1 (3/5) in stimulating rats to initiate copulation after the first ejaculatory sequence but this effect was not significant. 8-OHDPAT treated rats also ejaculated after fewer mounts and intromissions than rats treated with Compound 1. Mounts and intromissions preceding ejaculation were 0.7 ± 0.2 mounts and 5.5 ± 1.3 intromission for 8-OHDPAT treated rats whereas Compound 1 treated rats exhibited 9.4 ± 1.3 mounts and 15.2 ± 1.6 intromissions prior to ejaculation ($p < .001$ and $p < .002$ for mounts and intromissions respectively). Post-ejaculatory intervals were 637 (492-762) (range) seconds and 1030 ± 129 seconds for sexually exhausted rats receiving Compound 1 and 8-OHDPAT respectively.

Results from this Example represent the first report of the effects of an erectogenic arylpiperazine on copulatory function in exhausted male rats. In the present study Compound 1 significantly increased the number of sexually exhausted rats engaging in copulation and also significantly increased the numbers of these rats attaining ejaculation. These results suggest that Compound 1 has positive effects on motivational and desire aspects of copulatory function similar to, but less marked than, 8-OHDPAT. While no attempt was made to determine the nature of the stimulus effect of Compound 1 on copulation in sexually exhausted rats, the compound space required for the stimulus effects of a chemically diverse group of compounds may be expanded to include at least one form of arylpiperazine.

Based on results from *in vitro* experiments in isolated CC smooth muscle, which showed that Compound 1 failed to act as an α-adrenergic receptor antagonist, it can be concluded that, unlike yohimbine, the stimulus effects of Compound 1 in sexually exhausted rats are in no way related to direct blockade of α-adrenoceptors. At present there is limited information in the literature regarding the effects of serotonergic ligands other than 8-OHDPAT on sexual exhaustion. Yells et al. (1994) have demonstrated that the 5-HT re-uptake inhibitor fluoxetine produced limited, but significant, inhibitory effects on sexual response variables during the first ejaculatory series and markedly inhibited copulation during the last ejaculatory series prior to sexual exhaustion. Foreman et al. (1992a) have also demonstrated an

inhibitory effect for the 5-HT releaser fenfluramine on sexual response variables in normal rested rats. From the results obtained in the present and the previous sets of experiments it is suggested that the pharmacological profile for Compound 1 on sexual responses in normal and exhausted rats does not resemble those of a 5-HT re-uptake inhibitor or releaser.

The above examples showed that compounds of the present invention such as Compound 1 a) increase the appearance of ejaculation in the presence of erection b) enhance copulatory performance in rested and sexually exhausted rats mainly as a result of a reduction in ejaculatory threshold and an increase in the number of animals achieving ejaculation respectively and c) do not interact with 5-HT_{1B} receptors at maximally effective erectogenic doses and, unlike existing erectogenic arylpiperazines (e.g. m-CPP and TFMPP) which have affinity for and actions at these receptors, may be less likely to attenuate erectile responses induced by actions at 5-HT_{2C} and/or dopamine receptors..

In vitro experiments found that Compound 1 failed to relax pre-contracted corporal smooth muscle and also failed to enhance neurogenic/nitrergic relaxation thus suggesting that Compound 1, unlike the trazodone metabolite and erectogenic arylpiperazine m-CPP, may have less propensity to induce priapism in the presence of erection. Furthermore such a pharmacological profile also indicates that Compound 1 would be less likely to have adverse actions on other aspects of sexual function, such as ejaculation, which are dependent on functional α-adrenoceptors. The above examples showed that Compound 1, unlike m-CPP and TFMPP, both of which are known to possess α-adrenergic receptor blocking actions and agonist actions at 5-HT_{1B} receptors, enhanced ejaculation in the presence of erection.

Compound 1 enhanced rather than attenuated the ejaculatory performance of rested and sexually exhausted rats. Based on current knowledge regarding the ontogeny of these behavioral responses and by comparison with compounds known to affect these responses it is suggested that the stimulant actions of Compound 1 in these responses are not the result of blockade of serotonin uptake or enhanced serotonin release.

EXAMPLE 12

THE EFFECTS OF COMPOUND 1 ON SEROTONIN MEDIATED CONTRACTILE RESPONSES OF VASCULAR AND NON-VASCULAR SMOOTH MUSCLE FROM RATS.

Methods

Preliminary experiments were conducted in rat aorta and basilar artery in an attempt to differentiate possible effects on smooth muscle contractions elicited by potassium chloride (KCl), noradrenaline (NA) and serotonin (5-HT). Based on results obtained from these preliminary studies more detailed studies were conducted in rat stomach fundus (as a bioassay for 5-HT_{2B} receptor activity).

i) **Isolated Rat Aorta/Rat Basilar Artery**. Male SD rats (280-350 g) were sacrificed with CO_2 and the thoracic aorta removed and cleared of connective tissue. Four ring segments of 0.5 cm length were prepared from one aorta and suspended in separate 10 ml organ baths. Each tissue was connected to an isometric force-displacement transducer under a resting tension of 1 g. After one hour of equilibration in Carbogen® gassed Krebs-Henseleit buffer, with washings at 15 minute intervals, tissues were exposed to saline or Compound 1 (3×10^{-6} to 10^{-4} M) for 5 minutes before construction of concentration-response curves to increasing concentrations of KCl (5-80 mM), NA (10^{-9} to 10^{-4} M) or 5-HT (3×10^{-7} to 3×10^{-4} M). Increasing concentrations of contractile drugs were not added until plateau responses had been obtained to lower concentrations (approximately 1-3 minutes).

Rat basilar artery was obtained, prepared and treated as described for experiments with rat aorta. However in this series of experiments only two high concentrations of Compound 1 were tested against the contractile responses of 5-HT. Tissue preparations were incubated with saline or Compound 1 (10^{-5} or 10^{-4} M) for 5 minutes prior to construction of concentration-response curves for increasing concentrations of 5-HT (3×10^{-8} to 10^{-4} M). Data was calculated as the percentage of the maximal response to agonist in each tissue.

ii) **Isolated rat stomach fundus**. Male SD rats (500-700 g) were sacrificed with CO_2 and the fundus portion of the stomach was removed and prepared

as described previously (Clineschmidt et al., 1985, J. Pharmacol. Exp. Ther. 696). Briefly, the stomach from each animal was divided into two strips of equal length and width. Incisions were made perpendicular to the vertical plane of the muscle strip such that contractile force was transmitted along the vertical axis of the tissue. Tissues were prepared and allowed to equilibrate as described above.

Preparations were challenged with 5-HT (10^{-6} M) three times at hourly intervals with 5 exchanges of bathing fluid between tests. One hour after the last challenge with 5-HT, concentration-response curves were constructed for the contractile actions of 5-HT (10^{-10} to 10^{-4} M), Compound 1 (10^{-10} to 10^{-4} M) and m-CPP (10^{-10} to 10^{-4} M). Contractile responses to the three drugs were calculated as the percent of the maximal effect of the third challenge with 5-HT (10^{-6} M).

To compare the effects of Compound 1 and 1-naphthylpiperazine (1-NP) as antagonists at the stomach fundus receptor, individual strips were challenged twice with 5-HT as described above to confirm viability. After the last 5-HT challenge a complete concentration-response curve was constructed for 5-HT. Tissues were then incubated with saline, Compound 1 (10^{-8} to 10^{-4} M) or 1-NP (3×10^{-9} or 3×10^{-7} M) for 45 minutes prior to reconstruction of concentration-response curves for 5-HT. For all concentration-response curves 5-HT was added at 90 second intervals. Data was calculated as the percentage of the maximal response to 5-HT obtained for concentration-response curves prior to the addition of test drug.

All data were derived from four parameter logistic curve fits, of the form response = minimum + maximum-minimum / (1 + (dose / EC_{50}) ^ slope), to individual concentration-response curves. Values represent the mean ± SEM for the number of experiments indicated by n. Data were analysed by single factor ANOVA with Dunnett's test for multiple comparisons or students t-test with $p < 0.05$ established as the level of significance.

Results

i) Aorta/Basilar Artery. At the highest concentration tested Compound 1 (10^{-4} M) failed to significantly alter the contractile responses of rat aorta

to increasing concentrations of KCl (Figure 11). Maximal responses to KCl, EC_{50} and slope estimates were not significantly different in the presence of a high concentration of Compound 1 (Table 7).

Table 7

THE EFFECTS OF COMPOUND 1 ON KCL MEDIATED CONTRACTION OF RAT AORTA

	Pre Treatment		
Variable	Saline (0.9%)	Compound 1 (10^{-4} M)	t-test
Maximal Response (g)	0.86 ± 0.10	0.80 ± 0.10	P = 0.66
EC_{50} (mM)	14.9 ± 2.6	14.5 ± 1.9	P = 0.91
Slope (n)	4.3 ± 1.1	5.0 ± 1.0	P = 0.66

Table 7 shows the effects of Compound 1 on the concentration-response characteristics of KCl mediated contraction of isolated rat aorta. EC_{50} and slope values were derived from curve fits to individual concentration-response curves. Data are expressed as the mean ± SEM for 6 experiments. t-test indicates comparisons for significant differences between treatment groups for the variable indicated.

Compound 1 failed to significantly alter maximal responses or slope of the contractions elicited by NA (Figure 12). At the lowest concentration tested Compound 1 shifted the EC_{50} for NA mediated contraction in a leftward manner that approached, but did not breech, the established level of significance (Table 8).

Table 8

THE EFFECTS OF COMPOUND 1 ON NA MEDIATED CONTRACTION OF RAT AORTA

	Pre Treatment				
Variable	Saline (0.9%)	Compound 1 (10^{-5} M)	Compound 1 (3×10^{-4} M)	Compound 1 (10^{-4} M)	ANOVA
Maximal Response (g)	1.19 ± 0.38	1.10 ± 0.10	1.10 ± 0.05	1.24 ± 0.14	p = 0.89
EC_{50} (nM)	150 ± 80	50 ± 30	70 ± 20	80 ± 20	p = 0.05
Slope	1.19 ± 0.38	1.35 ± 0.54	1.43 ± 0.73	1.57 ± 0.70	p = 0.85

Table 8 shows the effects of Compound 1 on the concentration-response characteristics of NA mediated contraction of isolated rat aorta. EC_{50} and slope values were derived from curve fits to individual concentration-response curves. Data are expressed as the mean ± SEM for 4 experiments. ANOVA indicates comparisons for significant differences between treatment groups for the variable indicated.

In isolated rat aorta contracted with 5-HT, Compound 1 produced a concentration-dependent rightward shift of the concentration-response curve (Figure 13). At lower concentrations Compound 1 shifted the EC_{50} for 5-HT mediated contraction, in a non-significant trend, without affecting the slope of the relationship or maximal responses to 5-HT. At the highest concentration however Compound 1 significantly increased the EC_{50} for 5-HT mediated contraction without affecting the slope and maximal responses of the tissue preparations to 5-HT (Table 9).

Table 9

THE EFFECTS OF COMPOUND 1 ON 5-HT MEDIATED CONTRACTION OF RAT AORTA

	Pre Treatment (M)					
Variable	Saline (0.9%)	Compound 1 (3×10^{-6} M)	Compound 1 (10^{-5} M)	Compound 1 (3×10^{-4} M)	Compound 1 (10^{-4} M)	ANOVA
Maximal Response (g)	1.51 ± 0.12	1.39 ± 0.10	1.37 ± 0.14	1.38 ± 0.10	1.23 ± 0.14	p = 0.59
EC_{50} (μM)	9.32 ± 1.79	13.20 ± 1.47	13.0 ± 1.76	14.1 ± 2.67	43.0 ± 3.57*	p < 0.01
Slope	1.68 ± 0.17	1.48 ± 0.13	1.81 ± 0.08	1.62 ± 0.06	2.38 ± 0.44	p = 0.07

Table 9 shows the effects of Compound 1 on the concentration-response characteristics of 5-HT mediated contraction of isolated rat aorta. EC_{50} and slope values were derived from curve fits to individual concentration-response curves. Data are expressed as the mean ± SEM for 5-7 experiments. ANOVA indicates comparisons for significant differences between treatment groups for the variable indicated. An asterisk indicates a significant difference from saline controls.

Compound 1 had complex actions on rabbit basilar artery. At a concentration of 10 μM Compound 1 failed to shift the EC_{50} for 5-HT mediated contraction and also failed to alter the slope of the 5-HT concentration-response relationship. However at a concentration of 100 μM Compound 1 significantly increased the EC_{50} for 5-HT mediated contraction and reduced the slope of the concentration-response curve without affecting the maximal response to 5-HT (Figure 14; Table 10).

Table 10

THE EFFECTS OF COMPOUND 1 ON 5-HT MEDIATED CONTRACTION OF ISOLATED RAT BASILAR ARTERY

	Pre-treatment			
Variable	Saline (0.9%)	Compound 1 (10^{-5} M)	Compound 1 (10^{-4} M)	ANOVA
Maximal Response (g)	0.62 ± 0.05	0.52 ± 0.05	0.58 ± 0.04	P = 0.30
EC_{50} (μM)	0.14 ± 0.04	0.12 ± 0.04	0.77 ± 0.04*	p < 0.001
Slope (n)	0.79 ± 0.09	0.90 ± 0.12	0.40 ± 0.01*	p = 0.017

Table 10 shows the effects of Compound 1 and saline on the concentration-response characteristics of 5-HT mediated contraction of the isolated rat basilar artery. Maximal effect (g) is the maximum tension developed in grams whereas EC_{50} and slope are the curve-fit locator and descriptor as derived from averages of curve-fits to individual concentration-response curves. Data are expressed

as the mean ± SEM for 4-8 strips. An asterisk indicates significant differences between treatment groups for the variable indicated ($p < 0.05$ by ANOVA).

ii) **Fundus**. In rat stomach fundus 5-HT elicited a concentration-dependent contraction which was maximal at 100 μM. m-CPP behaved as a partial agonist with less maximal efficacy but similar potency to that of 5-HT (Figure 19, Table 11). Compound 1 failed to elicit contraction in a manner consistent with an agonist profile in this tissue. Compound 1 exhibited markedly less maximal efficacy and reduced potency compared to both 5-HT and m-CPP (Table 11).

Table 11

THE EFFECTS OF 5-HT, M-CPP AND COMPOUND 1 ON RAT STOMACH FUNDUS

	Test Drug			
Variable	5-HT	m-CPP	Compound 1	ANOVA
Maximal Effect (%)	91.9 ± 4.5+	36.4 ± 3.0*	10.1 ± 1.7*+	$p < 0.01$
EC_{50} (μM)	0.03 ± 0.02	0.08 ± 0.04	2.0 ± 1.7	$p = 0.27$
Relative Potency#	1.0	0.35	.013	

Table 11 shows the contractile effects of serotonin (5-HT), m-Chlorophenylpiperazine (m-CPP) and Compound 1 on isolated rat stomach fundus. Pre-drug maximal responses to the final test dose of 5-HT (1.0 μM) were not significantly different at 6.1 ± 0.6 g, 5.7 ± 0.4 g and 5.4 ± 0.6 g for 5-HT, Compound 1 and m-CPP respectively ($p > 0.70$ by ANOVA). An asterisk indicates a significant difference from the 5-HT treated tissues and + indicates a significant difference from m-CPP treated tissues (ANOVA and Dunnett's test; $p < 0.05$). # Calculated as the ratio of the EC_{50} (5-HT)/EC_{50} (Non-indole).

Increasing concentrations of Compound 1 (10^{-8} to 10^{-4} M) failed to significantly antagonize the fundus contractile response to 5-HT. Percent of maximal pre-drug effect, pD_2 and slope values for 5-HT mediated contraction in the presence of Compound 1 were not significantly different from the same values obtained in saline treated strips (Table 12). However, the slope values for 5-HT mediated contraction in

the presence of the low concentration of Compound 1 were significantly less than the slope value for 5-HT mediated contraction in the presence of 3×10^{-7} M 1-NP. 1-NP potently and competitively antagonized the fundus response to 5-HT by increasing the pD_2 estimate for half maximal responses without affecting the slope or maximum of the concentration-response relationship (Figure 16; Table 12).

Table 12

THE EFFECTS OF COMPOUND 1 AND 1-NAPHTHYLPIPERAZINE ON 5-HT MEDIATED CONTRACTIONS OF RAT STOMACH FUNDUS

	Tissue Response Variable		
Treatment	Maximum (%)	pD_2	Slope (n)
Saline	97 ± 7	8.0 ± 0.1	0.73 ± 0.10
Compound 1 10^{-4} M	113 ± 9	7.3 ± 0.2	0.58 ± 0.06
Compound 1 10^{-6} M	110 ± 5	7.3 ± 0.3	0.41 ± 0.05
Compound 1 10^{-8} M	107 ± 2	7.6 ± 0.5	0.38 ± 0.07
1-NP 3×10^{-9} M	98 ± 6	5.6 ± 0.3*	0.62 ± 0.08
1-NP 3×10^{-7} M	110 ± 6	4.7 ± 0.1*	1.99 ± 1.43
ANOVA (p)	$p > 0.40$	$p < 0.001$	$p < 0.05$

Table 12 shows the effects of increasing concentrations of Compound 1 and 1-naphthylpiperazine (1-NP) on 5-HT mediated contraction of the rat stomach fundus. Maximal pre-drug responses for determination of % maximal effect for 5-HT in the presence of saline (6.1 ± 0.4 g), Compound 1 (3.0 ± 0.5, 4.3 ± 0.3, 3.9 ± 0.7 g) and 1-NP (4.0 ± 0.8, 4.2 ± 0.6 g) were not significantly different ($p > 0.05$ by ANOVA). pD_2 was calculated as the negative logarithm of the EC_{50} from individual curve fits. Data are expressed as the mean ± SEM for 4-12 strips for the variable indicated in the table. An asterisk indicates a significant difference from saline treated strips whereas a + indicates a significant difference from strips treated with the highest concentration of 1-NP.

At a concentration of 100 µM Compound 1 failed to affect the contractile response of rat aorta to potassium chloride and noradrenaline. These

results suggest that Compound 1 lacks the ability to affect depolarization and calcium dependent contractions and also lacks α-adrenergic receptor antagonist actions in this preparation. The latter result is in keeping with an observed lack of α-adrenergic receptor antagonist action determined in rabbit and human corporal smooth muscle. Compound 1 did however produce a rightward shift in the concentration-response curve for 5-HT mediated contraction of this preparation. Previous work with selective 5-HT_{2A} agonists and antagonists have shown that the receptor mediating the contractile effects of 5-HT in the rat aorta is of the 5-HT_{2A} subtype (Ogawa et al., 1995, Jpn. Circ. J. 89; Kalkman & Schnieder, 1996, Pharmacol. 351). The antagonism of the 5-HT mediated contractile response by Compound 1 appeared to be competitive in nature as the EC_{50} was shifted in a parallel manner without a significant reduction in the maximal response obtained and without significant change in the slope of the concentration-response relationship. A competitive antagonist action has been demonstrated for several other arylpiperazines in rat jugular vein (Cohen & Fuller, 1983), a bioassay system believed to be responsive to the contractile effects of 5-HT through 5-HT_{2A} type serotonin receptors (Leysen et al., 1982). Taken together these results suggest that in the rat aorta, over the concentration range tested, Compound 1 behaves as a selective and competitive antagonist of 5-HT_{2A} receptors.

Rat basilar artery is commonly used as a preparation for the bioassay of 5-HT ligands (Skingle et al., 1996, Behav. Brain Res. 157). Numerous reports have suggested that multiple 5-HT receptor subtypes mediated contraction in the basilar artery of a number of species (Connor et al., 1989; Frenken, 1989; Gaw et al., 1990). The low concentration effect is believed to be mediated by a 5-HT_1 type receptor (Parsons & Whalley, 1989; Ohnuki & Ogawa, 1997) whereas at higher concentrations maximal responses are obtained with compounds having agonist activity at 5-HT_2 receptors, but not 5-$HT_{1D/1B}$ receptors (Martin et al., 1997, Br. J. Pharmacol. 157). In rat basilar artery maximal contractile responses are only observed with ligands which have agonist actions at 5-HT_2 receptors and the high dose component of the response to the endogenous agonist 5-HT is selectively abolished by antagonists of 5-HT_{2A} receptors (Deckert & Angus, 1992, Eur. J. Pharmacol. 17).

In the present study Compound 1 at a concentration of 10 μM failed to antagonize the contractile responses of rat basilar artery in the presence of increasing concentrations of 5-HT. However a 10 fold increase in the Compound 1 concentration produced a marked shift in the EC_{50} and reduction in slope, but not the maximum, of the 5-HT concentration-response relationship. A change in the slope of the concentration-response relationship for 5-HT might be expected in the presence of an antagonist that failed to inhibit the low concentration 5-HT_1 component but displaced the 5-HT_2 mediated high concentration component. Conversely a compound having antagonist actions at both the 5-HT_1 and 5-HT_2 receptors would be expected to shift the curve in parallel such that no change in slope would be observed.

Recently the 5-HT receptor mediating contraction of the rat stomach fundus was assigned to the 5-HT_{2B} subtype based on molecular, pharmacological and biochemical data (Hoyer et al., 1994, Pharmacol. Rev. 46 (2): 157). Compound 1 clearly failed to act as a potent or efficacious agonist in the rat fundus preparation thus demonstrating a lack of functional 5-HT_{2B} agonist action *in vitro*. These results are in agreement with earlier examples showing a putative 5-HT_{2C} receptor mediated erection enhancing response. These results are in line with previous work which demonstrated that the rat stomach fundus does not contain mRNA for the 5-HT_{2C} receptor (Baez et al., 1990; Foguet et al., 1992). Compound 1 also failed to act as an antagonist at fundus 5-HT_{2B} receptors. This lack of antagonist action was obvious when compared to the potent and competitive antagonism of 5-HT responses of these tissues in the presence of the non-selective 5-HT antagonist 1-NP.

The results obtained with vascular and non-vascular smooth muscle *in vitro* confirm a selective action of Compound 1 at 5-HT_2 receptors which are not of the 5-HT_{2B} subtype. While in earlier experiments Compound 1 was found to behave as a 5-HT_{2C} agonist, the results presented here strongly indicate that it also acts as a 5-HT_{2A} antagonist.

EXAMPLE 13

THE EFFECTS OF COMPOUND 1 ON 5-HT_{1A} RECEPTOR MEDIATED BEHAVIORAL SYNDROME AND CORE TEMPERATURE IN RATS *IN VIVO*

This example described the experiments carried out to determine if Compound 1 produces physiological and behavioral responses indicative of 5-HT_{1A} receptor activation at maximally effective erection enhancing doses using bioassays known to be indicative of 5-HT_{1A} receptor activation such as the serotonin "behavioral syndrome" (Green & Backus, 1990). This syndrome includes hind limb abduction, flat body posturing, lower lip retraction and forepaw treading.

Methods

i) **5-HT Behavioral Syndrome**. Male SD rats (300-360 g; n=10 per test group) were injected (i.p.) with increasing doses of saline (1.0 ml/kg), Compound 1 (4.0-64.0 mg/kg.), m-CPP (0.2-3.12 mg/kg) or TFMPP (0.2-3.12 mg/kg) and observed 25 minutes later for the occurrence of 5-HT mediated behaviors over a 5 minute observation period. Rats were observed for the appearance of 1) flat body posture (lying flat on the ventral surface), 2) hindlimb abduction (hindlimbs fully extended behind the animal), 3) forepaw treading (clockwise circular motion of the forelimbs in a seated position) and 4) lower lip retraction (retraction of the lower lip such that incisors are visible). Items 1-3 were scored according to their being absent (0), present (1), marked (2) or consistent (3). Item 4 was scored according to incisor visibility as not visible (0), partially visible (1), half visible (2), fully visible (3), or gum and incisor fully visible (4).

ii) **Core Temperature 1**. Male SD rats (600-940 g; n=5 per test group) were injected (i.p.) with saline, Compound 1 (1.0-64.0 mg/kg), or 8-OHDPAT (0.65 mg/kg) followed 15 minutes later by an injection of saline or 8-OHDPAT (0.65 mg/kg; i.p.). Rectal temperature was measured 20 minutes after the last drug administration by digital thermometer placed approximately 2-3 cm into the rectum.

iii) **Core Temperature 2**. Male SD rats (420-620 g; n=5-7 per test group) were injected (i.p.) with saline, Compound 1 (2.0-32.0 mg/kg) or 8-OHDPAT (0.65 mg/kg) followed 15 minutes later by an injection (i.p.) of saline or 8-OHDPAT (0.65 mg/kg). Rectal temperature was measured as described above.

Results

i) **5-HT Behavioral Syndrome**. Rats treated with saline failed to exhibit any signs of the 5-HT behavioral syndrome. In rats treated with Compound 1 hindlimb abduction and flat body posture were significantly increased, compared to saline treated controls, at doses of 32.0 and 64.0 mg/kg. Forepaw treading was also significantly increased but only at a dose of 64 mg/kg. Lower lip retraction was not significantly affected at any dose of Compound 1 (Table 13). m-CPP showed only a slight and insignificant increase in forepaw treading at the highest dose tested. All other components of the 5-HT behavioral syndrome were not observed in rats receiving m-CPP (Table 13). TFMPP failed to induce any of the components of the 5-HT behavioral syndrome (Table 13). 8-OHDPAT on the other hand significantly increased the appearance of all components of the behavioral syndrome at doses above 0.1 mg/kg (Table 13).

ii) **Core Temperature 1**. Systemic administration of 8-OHDPAT produced a marked and significant reduction of -1.5 ± 0.1 ° C in core temperature of rats maintained at high ambient temperature (Figure 17). Pre-drug temperatures were not different between treatment groups (38.1-38.6 ° C; p = 0.51 by ANOVA). Compound 1 at a dose of 64 mg/kg increased temperature by +1.0 ± 0.2 ° C, a significant effect compared to saline control rats which exhibited a slight reduction in core temperature of -0.06 ± 0.2 ° C. However 4/5 of the Compound 1 treated animals exhibited convulsions and 1/5 of the animals died. Increasing doses of Compound 1 (1.0-64.0 mg/kg) produced a trend for reduction in the hypothermic effect of 8-OHDPAT with a significant reduction of the 8-OHDPAT induced hypothermia at a dose of 64 mg/kg Compound 1 (Figure 17). None of the rats receiving both the high dose of Compound 1 and 8-OHDPAT exhibited convulsions and none of the animals died.

Table 13

EFFECTS OF COMPOUND 1, TFMPP, M-CPP AND 8-OHDPAT ON THE APPEARANCE OF 5-HT BEHAVIORAL SYNDROME IN RATS

	Variable			
Treatment	HLA	FBP	FPT	LLR
Saline (1.0 ml/kg)	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
Compound 1 (mg/kg)				
4	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
8	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.1
16	0 ± 0	0.3 ± 0.2	0 ± 0	0.2 ± 0.1
32	0.9 ± 0.2*	1.5 ± 0.2*	0 ± 0	0.4 ± 0.2
64	2.2 ± 0.1*	2.1 ± 0.1*	0.5 ± 0.2*	0.6 ± 0.2
m-CPP (mg/kg)				
0.2	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
0.5	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
0.75	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.2
1.25	0 ± 0	0.1 ± 0.1	0 ± 0	0.3 ± 0.2
3.12	0 ± 0	0 ± 0	0.3 ± 0.2*	0.5 ± 0.2
TFMPP (mg/kg)				
0.2	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.2
0.5	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.2
1.0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
1.25	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.2
3.12	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.2
8-OHDPAT (mg/kg)				
0.1	0 ± 0	0 ± 0	0.5 ± 0.2	0.4 ± 0.2
0.3	0.8 ± 0.1*	1.1 ± 0.2*	0.6 ± 0.2*	2.2 ± 0.3*
1.0	1.5 ± 0.3*	1.6 ± 0.2*	1.8 ± 0.2*	2.9 ± 0.4*
3.0	2.1 ± 0.3*	2.1 ± 0.3*	2.7 ± 0.2*	3.6 ± 0.2*

Table 13 shows the effects of increasing doses of Compound 1, m-CPP, TFMPP, 8-OHDPAT and saline on the appearance of 4 components of the 5-HT behavioral syndrome. Hindlimb abduction (HLA), flat body posture (FBP), forepaw treading (FPT) and lower lip retraction (LLR) were scored over 5 minutes as a maximum of 3 or 4 as described in the methods.

iii) Core Temperature 2. A second set of experiments was run to determine the effects of lower doses of Compound 1 alone and, a lower non-convulsive dose of Compound 1 in combination with 8-OHDPAT on core temperature. Pre-drug temperature values were not significantly different between treatment groups (39.1-39.6 ° C; p = 0.59 by ANOVA). Saline treated animals displayed an increase in core temperature of +0.4 ± 0.4 ° C whereas those receiving 8-OHDPAT, as demonstrated above, showed a marked and significant hypothermia of -1.1 ± 0.1 ° C (Figure 18). Increasing doses of Compound 1 (2.0-32.0 mg/kg) produced a reduction in core temperature but this effect was not significantly different from saline treated animals and did not appear to be dose-related. Compound 1 at a dose of 32.0 mg/kg reduced the hypothermic effect caused by 8-OHDPAT but this effect failed to reach significance (Figure 18).

5-HT1$_A$ receptors are known to exist as pre-synaptic somatodendritic receptors and post-synaptic receptors on serotonergic neurones and heteroreceptors on non-serotonergic nerve fibers (Fletcher et al., 1993, Trends Pharmacol. Sci. 41; Hoyer & Boddeke, 1993). Therefore activation of any one or a number of these receptors by non-selective drugs may be expected to produce a broad range of effects based on a summation of the different physiological responses that are activated or inhibited. In rats selective 5-HT$_{1A}$ agonists and partial agonists elicit an aggregate of behaviors which is known as the 5-HT behavioral syndrome (Tricklebank et al., 1984, Eur. J. Pharmacol. 271; Green & Heal, 1985). Of the components of this syndrome forepaw treading, hypothermia and lower lip retraction are known to be mediated by post-synaptic receptors since these effects are not attenuated by destruction of the serotonergic system with either pCPA or 5,7-DHT (Heal et al., 1989) and are

antagonized by selective and non-selective 5-HT_{1A} antagonists (Nisbet & Marsden, 1984; Sanchez et al., 1996, Eur. J. Pharmacol. 245).

In the present study the full 5-HT_{1A} agonist 8-OHDPAT was very potent in eliciting all of the components of the 5-HT behavioral syndrome. m-CPP and TFMPP on the other hand were not potent or effective with respect to initiation of any of the components of the behavioral syndrome. These results agree with previous studies which show that both of latter compounds only induce signs of the behavioral syndrome at toxic doses where penile erections are replaced by forepaw treading (Lucki et al., 1989; Berendsen & Broekkamp, 1987, Eur. J. 279).

The effects of Compound 1 on the components of the behavioral syndrome are consistent with results obtained in previous sections that suggested activity at 5-HT_{1A} receptors (hypothermia and improved copulatory performance). However the appearance of a significant effect on the components of the behavioral syndrome in the absence of toxic effects (convulsions) was only obtained at a dose of 32 mg/kg. In the example demonstrating the actions of Compound 1 on copulation, it was shown that a dose of 16 mg/kg facilitated copulation in normal and sexually exhausted rats, a dose which was without significant effect on components of the 5-HT behavioral syndrome. This apparent corollary suggests that it is not full activation of 5-HT_{1A} receptors which is responsible for the positive effects on copulation.

The second set of experiments conducted in the present study was designed to determine if Compound 1 acts as a partial agonist at the same receptors which mediate the 8-OHDPAT induced hypothermia (post-synaptic 5-HT_{1A} receptors). A full agonist would elicit a maximal hypothermic response but would also fail to prevent the hypothermia induced by 8-OHDPAT. A partial agonist on the other hand would be expected to elicit a submaximal hypothermic response and also prevent the expression of maximal hypothermia produced by the full agonist 8-OHDPAT (Hoyer & Boddeke, 1993). Increasing doses of Compound 1 produced a significant dose-related reduction in the hypothermic response to a standard dose of 8-OHDPAT. However Compound 1 alone, at a dose of 64.0 mg/kg, produced a marked and significant hyperthermia such that the effects on the 8-OHDPAT hypothermia

may have been the result of a functional antagonism mediated by a different receptor subtype rather than a true pharmacological antagonism of the 8-OHDPAT-5-HT_{1A} receptor mediated response.

A number of other 5-HT_{2C} agonists and indirect acting serotonin agonists have also been shown to induce marked hyperthermic responses when administered systemically at high doses (Wiczyńska et al., 1989a, 1989b; Mazzola-Pomietto et al., 1996; Mazzola-Pomietto et al., 1997, Psychopharmacol. 144). Therefore it is possible that the effects of Compound 1 on the 8-OHDPAT induced hypothermia are mediated by activation of 5-$HT_{2A/2C}$ receptors. However Compound 1 antagonized 5-HT_{2A} receptors *in vitro* thus suggesting that the hyperthermic effect observed here is due to 5-HT_{2C} receptor agonist action.

The third set of experiments was performed to profile the dose-response relationship for hypo-and/or hyperthermia caused by Compound 1 and the interaction of the highest non-convulsive dose of Compound 1 with 8-OHDPAT. Compound 1 at lower doses elicited a hypothermia compared to saline controls which did not appear to be dose-related. Although the highest non-convulsive dose of Compound 1 did itself cause a hypothermia and attenuated the hypothermia induced by 8-OHDPAT this effect failed to reach the required level of significance. Furthermore the slight hypothermia induced by 2.0 and 8.0 mg/kg doses of Compound 1 also failed to reach significance compared to changes in core temperature in control animals. These equivocal results may be explained by a mixed functional antagonism between activation of both 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors mediating hypothermia and hyperthermia respectively.

The results presented here suggest that at lower doses Compound 1 may behave as a very weak 5-HT_{1A} partial agonist and at higher doses 5-HT_2 receptors mediating hyperthermia are activated. Based on the previous results obtained with Compound 1 in the presence of pCPA pre-treatment, it is suggested that the hyperthermic actions of high doses of Compound 1 are mediated by agonist actions at post-synaptic 5-HT_{2C}.

EXAMPLE 14

THE EFFECTS OF COMPOUND 1 ON DOI INDUCED HEAD-TWITCH IN RATS.

In earlier examples it was shown that one of the main factors responsible for the erection enhancing actions of Compound 1 was due to agonist action at post-synaptic 5-HT_{2C} receptors. In tests of copulatory function, 5-HT behavioral syndrome and hypothermia Compound 1 exhibited a pharmacological profile indicative of 5-HT_{2C} agonist. In *in vitro* smooth muscle tests Compound 1 appeared to behave as an antagonist at vascular 5-HT_2 receptors that were not of the 5-HT_{2B} subtype. Very early in the study of the physiological effects of 5-HT it was determined that hallucinogenic 5-HT agonists elicited a characteristic head-twitch response (Corne & Pickering, 1967, Psychopharmacol. 65). More recently this response has been characterized as an example of 5-HT_{2A} receptor activation (Green & Backus, 1990). A number of indirect and direct acting 5-HT agonists are capable of eliciting this response (Malick et al., 1976; Darmani et al., 1990a; Schreiber et al., 1995, J. Pharmacol. Exp. Ther. 101). Current research supports the idea that head-twitch responses caused by both indirect and direct acting 5-HT agonists are mediated by the 5-HT_{2A} receptor subtype. Results with antagonists have also demonstrated that compounds selective for 5-HT_{2A} receptors exhibit a strong relationship ($r > 0.80$) between binding affinity at 5-HT_{2A} sites and doses inhibiting DOI mediated head-twitch response in rats (Schreiber et al., 1995, J. Pharmacol. Exp. Ther. 101).

In this example, a rat model of DOI induced head-twitch was used to determine the activity of Compound 1 at 5-HT_{2A} receptors *in vivo*.

Methods

Male SD rats weighing 400-600 grams (n=5 per test group) were injected (s.c.) with saline, Compound 1 (2.0 , 8.0 or 32 mg/kg) or ketanserin at a dose of 1.0 mg/kg, and challenged 15 minutes later with a 2.5 mg/kg dose of DOI (i.p). The number of head-twitch responses occurring between 5 and 15 minutes after DOI administration were recorded (Schreiber et al., 1995, J. Pharmacol. Exp. Ther. 101). Data were

analysed by ANOVA with Dunnett's test for multiple comparisons and are expressed as the mean ± SEM for 5 animals per test group.

Results

In rats Compound 1 was able to inhibit head-twitch responses elicited by DOI in a dose-dependent manner. Compound 1 was as efficacious as ketanserin but less potent in its effects on DOI induced head-twitch (Figure 19). At the medium and highest doses of Compound 1 (8.0 & 32.0 mg/kg) the DOI head-twitch response was not significantly different from animals receiving only saline but was significantly less than DOI induced head-twitch response in the presence of saline. Although the DOI response in the presence of 2.0 mg/kg Compound 1 was not significantly different from animals receiving only saline, this response was also not significantly reduced compared to animals receiving DOI in the presence of saline.

The results obtained with Compound 1 suggest that the compound acts as an antagonist of 5-HT_{2A} receptors in the rat. Furthermore Compound 1 failed to induce head-twitch on its own provided additional support.

EXAMPLE 15

THE EFFECTS OF COMPOUND 1 ON 5-HT MEDIATED BRADYCARDIA IN RATS

Arylpiperazines have previously been assessed for their ability to bind at 5-HT_3 sites (Glennon et al., 1989; Anzini et al., 1995). Although a number of the arylpiperazines tested were shown to have reasonable affinity for the 5-HT_3 sites, very little information is available regarding their effects *in vivo* using functional bioassays. In light of the fact that compounds examined in this study all produced bell-shaped dose-response curves for erection, and that some 5-HT_3 receptor antagonists have been reported to reduce erection, the aim of the present experiment was to compare and contrast the actions of Compound 1 with other arylpiperazines (e.g. m-CPP and TFMPP) in an *in vivo* assay of 5-HT_3 receptor activation, the von Bezhold-Jarisch reflex.

Methods

Male SD rats (350-500 g) were anesthetized with urethane (1.5 g/kg i.p.) and the right external jugular vein and the left common carotid artery were cannulated (PE 50 polypropylene tubing) for drug administration and blood pressure recording respectively. ECG was monitored and recorded from limb leads in a lead II configuration. The animals trachea was cannulated (14G Jelco intracath) to facilitate ventilation with room air (Ugo Basile respirator; 10 ml/kg; intrinsic rate).

Prior to administration of test compounds a dose-response curve (1-30 µg/kg) for the bradycardic effects of 5-HT was constructed in each animal. Following this a dose producing submaximal bradycardia was tested three times at 10 minute intervals or until three stable responses were obtained. This dose then served as the control dose of 5-HT. Compound 1 (1.0-10.0 mg/kg; n=6), TFMPP (1.0-10.0 mg/kg; n=6), m-CPP (1.0-10.0 mg/kg; n=6), quipazine (0.001-0.1 mg/kg; n=6) or saline (1.0 ml/kg; n=6) were administered as single bolus doses, 0.1 ml/kg body weight, at 15 minute intervals. Responses to control doses of 5-HT were determined 5 minutes after the administration of test compounds. Ten minutes after the last control dose of 5-HT atropine (600 µg/kg) was administered and the effects of the control dose of 5-HT were observed 5 minutes later.

Changes in heart rate before and after 5-HT and test drug administration were calculated from the R-R interval of the ECG. Data for individual dose-response curves were compared by ANOVA whereas comparisons for the effects of 5-HT pre- and post atropine were compared by t-test with $p < 0.05$ taken as a significant result.

Results

5-HT induced a dose-dependent bradycardia as an i.v bolus dose between 1-30 µg/kg. In preliminary tests doses above 100 µg/kg always produced A-V block such that 30 µg/kg was the maximum dose used in drug experiments. Individual rats responded differently to 5-HT such that different doses were used in different animals to produce a similar bradycardia. The median effective control dose

for each group was between 1-30 μg/kg and produced bradycardia that was not significantly different between test groups. 5-HT induced bradycardia and median effective doses (in parentheses) were 108 ± 10 (10 μg/kg), 101 ± 13 (30 μg/kg), 124 ± 6 (30 μg/kg), 109 ± 6 (10 μg/kg) and 114 ± 7 (10 μg/kg) beats per minute for Compound 1, quipazine, m-CPP, TFMPP and saline treated groups respectively ($p > 0.50$).

Saline also failed to reduce the effects of 5-HT with a maximal reduction of only 12 beats per minute in the 5-HT mediated bradycardia. Compound 1 (0.1-10.0 mg/kg) and TFMPP (0.1-10 mg/kg) also failed to antagonize the bradycardia elicited by control doses of 5-HT in urethane anesthetized rats (Figure 20). Compound 1 and TFMPP produced a maximal reduction in the 5-HT mediated bradycardia of only 11 and 6 beats per minute respectively. m-CPP (0.1-10 mg/kg) and quipazine (0.001-.1 mg/kg) on the other hand produced a dose-dependent reduction in the bradycardic response elicited by control doses of 5-HT (Figure 20). The inhibition of 5-HT mediated bradycardia was complete (100%) for m-CPP whereas quipazine mediated inhibition of the response was approximately 50% at the highest dose tested.

In the presence of atropine the 5-HT mediated bradycardia was significantly reduced in all treatment groups (data not shown). However due to the fact that at the end of experiments high doses of antagonists would have been present the effects of atropine become difficult to differentiate from antagonist actions. Therefore only the atropine responses in treated animals that did not show an antagonism of the 5-HT response are presented. In saline treated animals pre-drug responses to 5-HT induced bradycardia of 114 ± 7 beats per minute whereas in the presence of atropine (600 μg/kg) the response to 5-HT was diminished to 45 ± 6 beats per minute ($p < 0.05$; t-test). Similar results were obtained in Compound 1 and TFMPP treated animals with an atropine induced attenuation in the bradycardic actions of 5-HT from pre-drug values of 108 ± 10 and 109 ± 6 beats per minute respectively to 47 ± 14 and 50 ± 11 beats per minute respectively ($p < 0.05$; t-tests).

While both m-CPP and Compound 1 caused bradycardia at the highest doses tested only m-CPP was found to dose-dependently reduce the bradycardic response elicited by 5-HT. These results are in agreement with other studies using m-CPP *in vivo* (Robertson et al., 1992, Life Sci. 599). These results also demonstrate a distinct absence of 5-HT_3 receptor antagonist action for Compound 1 over the dose range studied. Compound 1 induced bradycardia prevented exploration of doses greater than those producing maximally effective erection enhancing actions in conscious rats such that it is possible that the compound may have 5-HT_3 antagonist actions at doses which show diminished erection enhancing effects. This together with the results from the affinity binding experiments (Example 5) may indicate Compound 1 provides a neutral activity at the 5-HT_3 receptor in the above model.

Results of the above examples demonstrated that compounds of the invention such as Compound 1 possess a unique combination of effects on sexual function related to their ability to interact differentially with several types of serotonin receptors. These findings include: a) compounds of the invention elicited erection in rats in a manner dependent upon agonist action at post-synaptic 5-HT_{2C} receptors within the CNS; b) compounds of the invention enhanced low grade erection and affiliative behaviors in non-human primates, c) compounds of the invention did not alter other central and peripheral pathways of erection; this was due to a lack of affinity for other serotonergic receptors and adrenergic receptors and d) compounds of the invention enhanced copulatory performance in normal and sexually exhausted male rats. The effects of compounds of the invention on copulatory performance differed considerably from the copulatory profiles previously reported for m-CPP and TFMPP. It was also demonstrated that: a) compounds of the invention selectively inhibited vascular smooth muscle contractions mediated by 5-HT through an apparent antagonism of 5-HT_{2A} receptors, b) compounds of the invention failed to act as an agonist or antagonist at 5-HT_{2B} receptors in the rats stomach fundus, c) compounds of the invention elicited several components of the 5-HT behavioral syndrome but only at toxic doses suggesting an activity as a partial agonist at 5-HT_{1A} receptors d) the effects of compounds of the invention on core temperature are either the result of its

activity as a partial agonist at 5-HT_{1A} receptors or agonist activity at 5-HT_{2C} receptors, e) compounds of the invention has actions in a rat model of DOI induced head-twitch which confirm its antagonist effect at 5-HT_{2A} receptors and f) compounds of the invention failed to antagonize 5-HT_3 receptors *in vivo* in dose ranges related to its maximal effects on erection.

While there are numerous reports of compounds having mixed agonist/antagonist profiles at other 5-HT receptors, 5-HT_{2A} and 5-HT_{2C} receptors exhibit close structural and pharmacological similarities such that it is difficult to envision a compound having the ability to act as an agonist at one of these receptors and an antagonist at the other. Therefore it is surprising to discover that compounds of the invention possess these properties. It is likely that compounds of the invention are uniquely able to adopt conformations which confer the correct combination of selective agonist, antagonist or neutral actions at specific receptors.

Compounds which enhance erection and improve copulatory performance should have broad application in the treatment of a wide variety of erectile and sexual dysfunctions.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually incorporated by reference. From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

1. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient.

2. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient.

3. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient.

4. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for the treatment or prevention of sexual dysfunction in a patient.

5. The use of claim 1, 2, 3 or 4 wherein the sexual dysfunction is male erectile dysfunction.

6. The use of claim 1, 2, 3 or 4 wherein the sexual dysfunction is impotence.

7. The use of claim 1, 2, 3 or 4 wherein the sexual dysfunction is female sexual arousal disorder and/or female inhibited orgasm.

8. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for increasing the libido of a male or female patient.

9. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for increasing the libido of a male or female patient.

10. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for increasing the libido of a male or female patient.

11. The use of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for increasing the libido of a male or female patient.

12. The use of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient.

13. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient.

14. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient.

15. The use of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}, for the manufacture of a medicament for enhancing the sexual performance of a male or female patient.

16. The use of claim 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds, provides a pro-erectile response in the patient.

17. The use of claim 1, 8 or 12 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

18. The use of claim 2, 9 or 13 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

19. The use of claim 3, 10 or 14 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

20. The use of claim 4, 11 or 15 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

21. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds is formulated for oral administration.

22. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds is formulated for topical administration.

23. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds is formulated for direct injection.

24. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds is formulated for one of intrameatal, intracavernous or intraurethral administration.

25. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14 or 15 wherein the compound or the combination of two or more compounds is formulated as a tablet with a disintegration time of less than one hour.

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

27. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3.

28. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}.

29. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

30. A pharmaceutical composition according to claim 26, 27, 28 or 29 wherein the composition is in the form of a tablet for oral administration, and the tablet has a disintegration time of less than one hour.

31. A method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

32. A method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3.

33. A method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}.

34. A method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

35. A method for treating or preventing sexual dysfunction in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a pharmaceutical composition according to claim 26, 27, 28, 29 or 30.

36. The method of claim 31, 32, 33, 34 or 35 wherein the sexual dysfunction is male erectile dysfunction.

37. The method of claim 31, 32, 33, 34 or 35 wherein the sexual dysfunction is impotence.

38. The method of claim 31, 32, 33, 34 or 35 wherein the sexual dysfunction is female sexual arousal disorder and/or female inhibited orgasm.

39. A method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

40. A method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3.

41. A method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}.

42. A method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

43. A method for increasing the libido of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a pharmaceutical composition according to claim 26, 27, 28, 29 or 30.

44. A method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds, that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A}.

45. A method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3.

46. A method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A}.

47. A method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds that can occupy the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A}.

48. A method for enhancing the sexual performance of a male or female patient, comprising administering to the patient in need thereof a therapeutically effective dose of a pharmaceutical composition according to claim 26, 27, 28, 29 or 30.

49. The method of claim 44, 45, 46, 47 or 48 wherein the compound or the combination of two or more compounds or the pharmaceutical composition, provides a pro-erectile response in the patient.

50. The method of claim 31, 39 or 44 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

51. The method of claim 32, 40 or 45 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

52. The method of claim 33, 41 or 46 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

53. The method of claim 34, 42 or 47 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

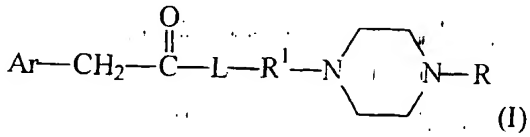
54. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 wherein the administration is by oral administration.

55. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 wherein the administration is by topical administration.

56. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 wherein the administration is by direct injection.

57. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47 or 48 wherein the administration is by one of intrameatal, intracavernous or intraurethral administration.

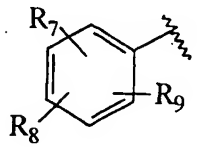
58. A method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C} and 5-HT_{2A} in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound, or a combination of two or more compounds of the formula (I)



(I)

including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

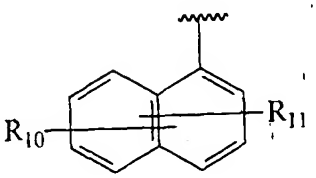
Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):



(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy,

C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and $N(R_{15},R_{16})$ where R_{15} and R_{16}, are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



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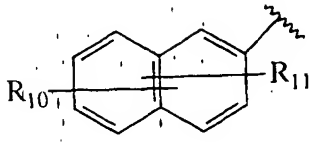
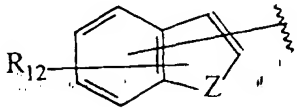


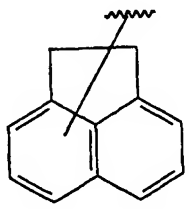
(III) (IV)

where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



(V)

where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and $N(R_{15},R_{16})$ where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-CH_2C(O)-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



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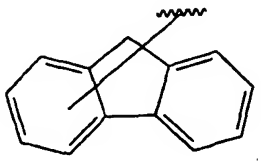


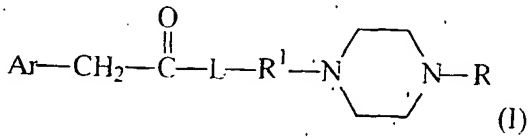
(VI) (VII);

L is selected from the group of a direct bond, O, NH, and N(C_1-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

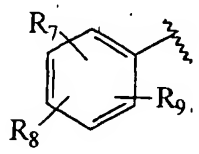
59. A method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_3 in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I)



(I)

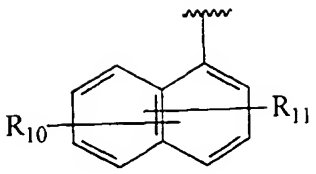
including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):

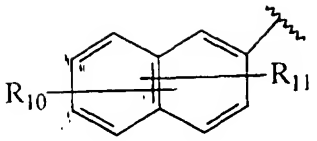


(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;

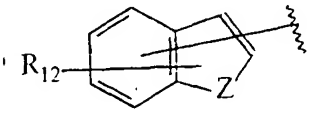


and



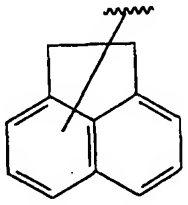
(III) (IV)

where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;

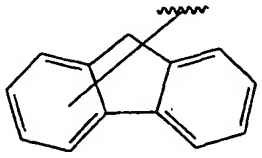


(V)

where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-CH_2C(O)-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



and



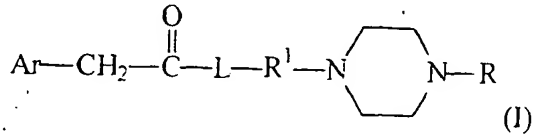
(VI) (VII);

L is selected from the group of a direct bond, O, NH, and N(C_1-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

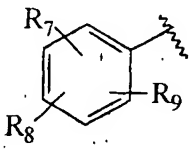
60. A method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A} and 5-HT_{1A} in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I)



(I)

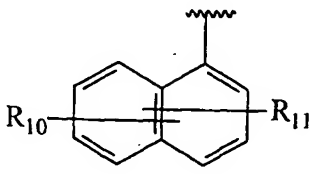
including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):



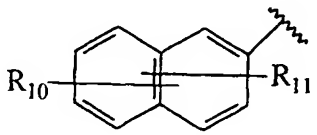
(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



(III)

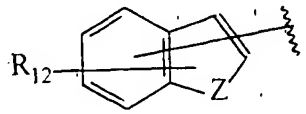
and



(IV)

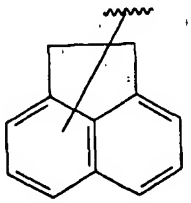
where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro,

sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



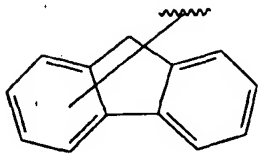
(V)

where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-CH_2C(O)-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



(VI)

and



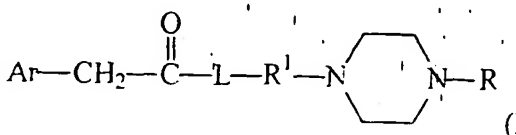
(VII);

L is selected from the group of a direct bond, O, NH, and N(C_1-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

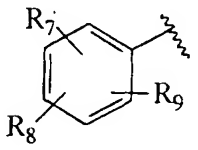
61. A method of occupying the following serotonin (5-HT) receptors: 5-HT_{2C}, 5-HT_{2A}, 5-HT_3 and 5-HT_{1A} in a patient, comprising administering to the patient in need thereof a therapeutically effective dose of a compound or a combination of two or more compounds of the formula (I)



(I)

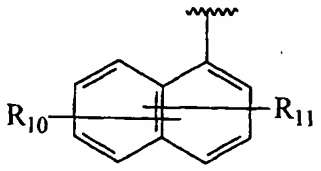
including salts, solvates, isolated enantiomers, isolated diastereomers, isolated tautomers, and mixtures thereof, wherein, independently at each occurrence:

Ar is selected from a C_3-C_{13}carbocyclic ring, a heteroaryl group, and ring systems selected from formulae (II), (III), (IV), (V), (VI), and (VII):

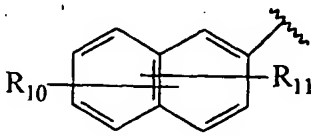


(II)

where R_7, R_8 and R_9 are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen (H), hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, aryl and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



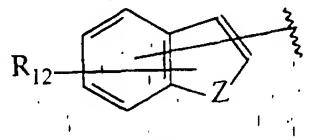
and



(III) (IV)

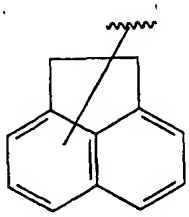
where R_{10} and R_{11} are independently selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy,

C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl;



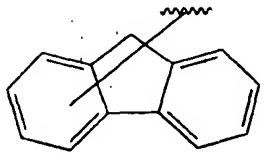
(V)

where R_{12} is selected from bromine, chlorine, fluorine, carboxy, hydrogen, hydroxy, hydroxymethyl, methanesulfonamido, nitro, sulfamyl, trifluoromethyl, C_2-C_7alkanoyloxy, C_1-C_6alkyl, C_1-C_6alkoxy, C_2-C_7alkoxycarbonyl, C_1-C_6thioalkyl, and N(R_{15},R_{16}) where R_{15} and R_{16} are independently selected from hydrogen, acetyl, methanesulfonyl, and C_1-C_6alkyl; and Z is selected from CH_2, O, N and S, where Z may be directly bonded to "-CH_2C(O)-L-" as shown in formula (I) when Z is N, or Z may be directly bonded to R_{17} when Z is N, and R_{17} is selected from hydrogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, aryl and benzyl; and



(VI)

and



(VII);

L is selected from the group of a direct bond, O, NH, and N(C_1-C_6alkyl);

R^1 is selected from the group of a direct bond, a C_1-C_6alkylene group, and a 1,2-disubstituted C_5-C_6cycloalkyl; and

R is selected from the group of H, a C_1-C_6alkyl and a C_7-C_{13}aralkyl.

62. The method of claim 58 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor and antagonist activity at the 5-HT_{2A} receptor.

63. The method of claim 59 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and neutral or agonist or antagonist activity at the 5-HT_3 receptor.

64. The method of claim 60 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor and partial agonist activity at the 5-HT_{1A} receptor.

65. The method of claim 61 wherein the compound or the combination of two or more compounds, provides agonist activity at the 5-HT_{2C} receptor, antagonist activity at the 5-HT_{2A} receptor, neutral or agonist or antagonist activity at the 5-HT_3 receptor and partial agonist activity at the 5-HT_{1A} receptor.

66. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 or 20 wherein the compound or the combination of two or more compounds does not interact with the alpha-adrenoceptors.

67. The use of claim 1, 2, 3, 4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 or 20 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

68. The use of claim 66 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

69. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52 or 53 wherein the compound or the combination of two or more compounds does not interact with the alpha-adrenoceptors.

70. The method of claim 31, 32, 33, 34, 35, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52 or 53 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

71. The method of claim 69 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

72. The method of claim 58, 59, 60, 61, 62, 63, 64 or 65 wherein the compound or the combination of two or more compounds does not interact with the alpha-adrenoceptors.

73. The method of claim 58, 59, 60, 61, 62, 63, 64 or 65 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

74. The method of claim 72 wherein the compound or the combination of two or more compounds does not interact with the 5-HT_{1B} receptor and/or 5-HT_{2B} receptor.

75. A method for screening test compounds for pro-erectile activity comprising:

(a) contacting a test compound with a serotonin 5-HT_{2C} receptor, and measuring the binding of the compound to the 5-HT_{2C} receptor; and

(b) contacting the test compound with a serotonin 5-HT_{2A} receptor, and measuring the binding of the test compound to the 5-HT_{2A} receptor.

76. A method according to claim 75 further comprising:

(c) contacting the test compound with a serotonin 5-HT_3 receptor, and measuring the binding of the test compound to the 5-HT_3 receptor.

77. A method according to claims 75 or 76 wherein the binding is measured by the %inhibition by the test compound on the binding of specific radioligand to the respective 5-HT subtype receptors (*e.g.*, [3H]-Ketanserin for 5-HT_{2A}; [3H]-Mesulergine for 5-HT_{2C}; and [3H]-GR65630 for 5-HT_3).

78. A method according to claims 77 wherein the %inhibition is at least 30% and preferably 50% or more.

79. A method according to claims 75 or 76 wherein the compound is a piperazine derivative.

Figure 1

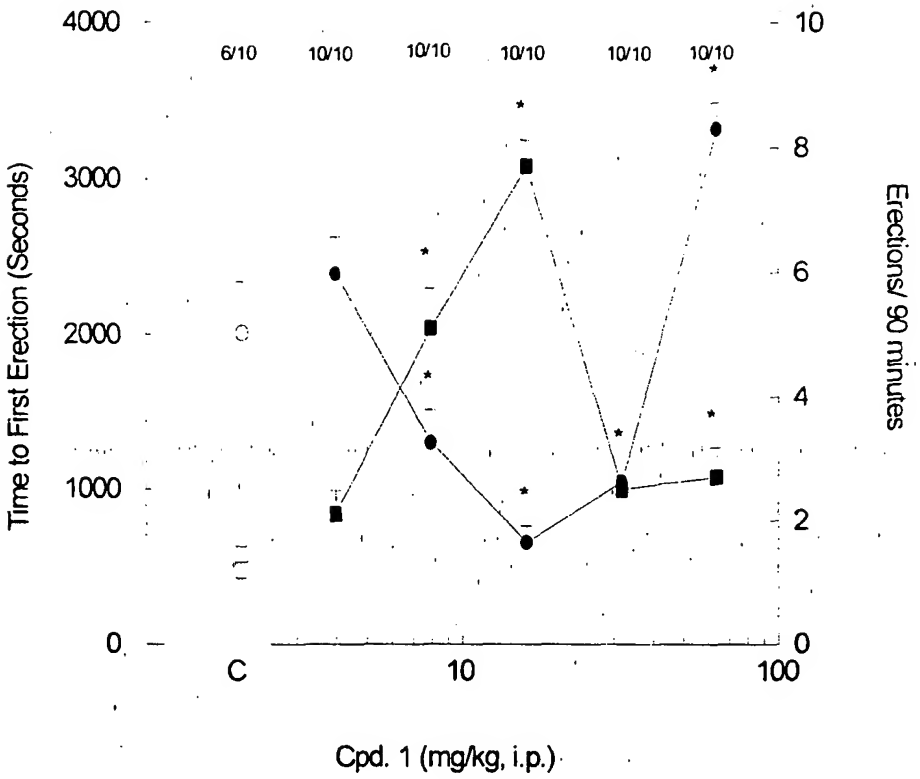

4000
3000
2000
1000
0
Time to First Erection (Seconds)
10
8
6
4
2
0
Erections/ 90 minutes
6/10
10/10
10/10
10/10
10/10
10/10
C
10
100
Cpd. 1 (mg/kg, i.p.)

Figure 2

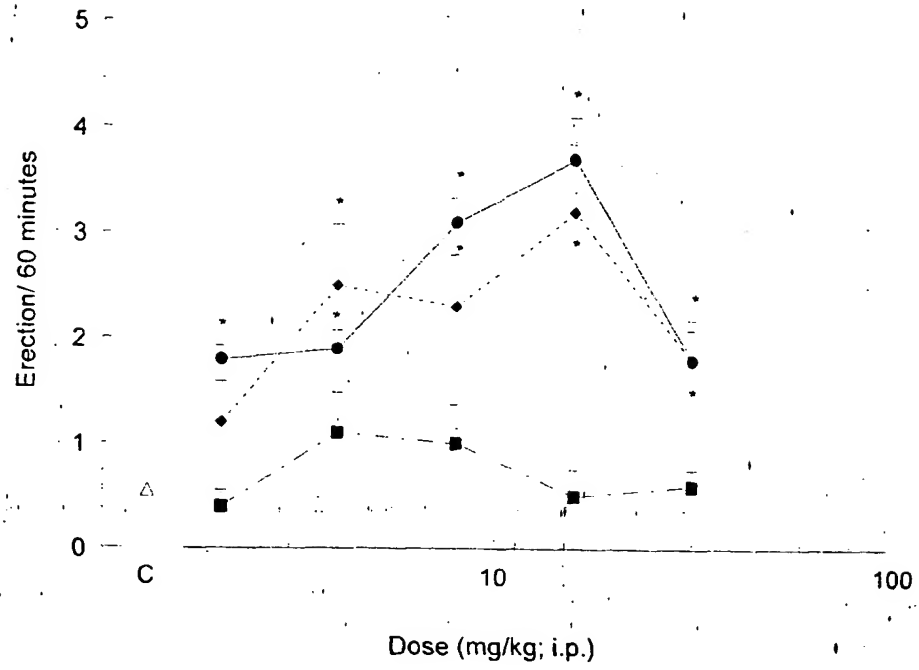


Figure 3

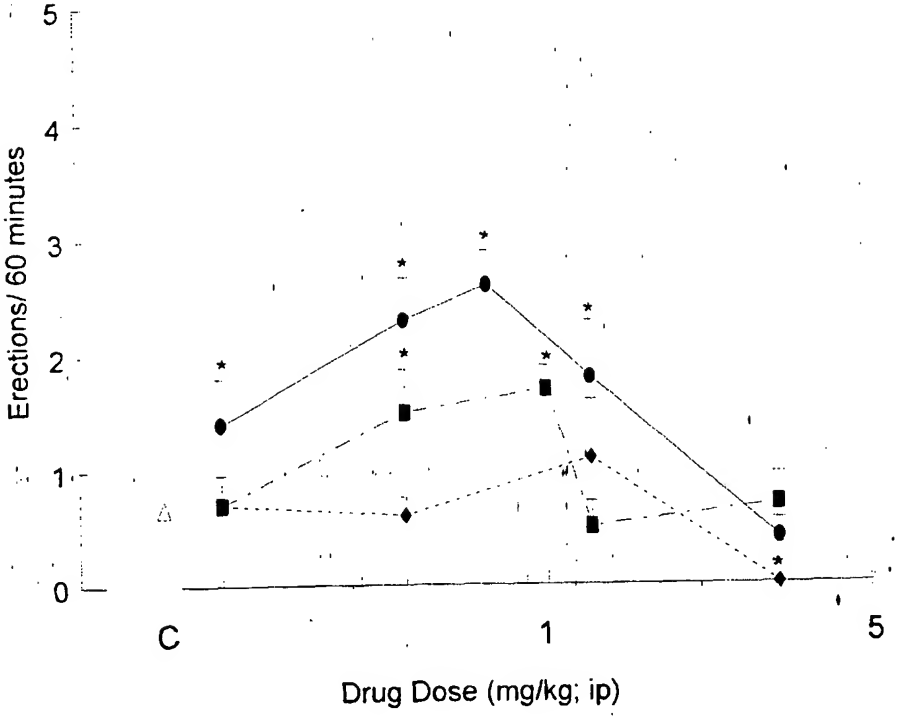


Figure 4

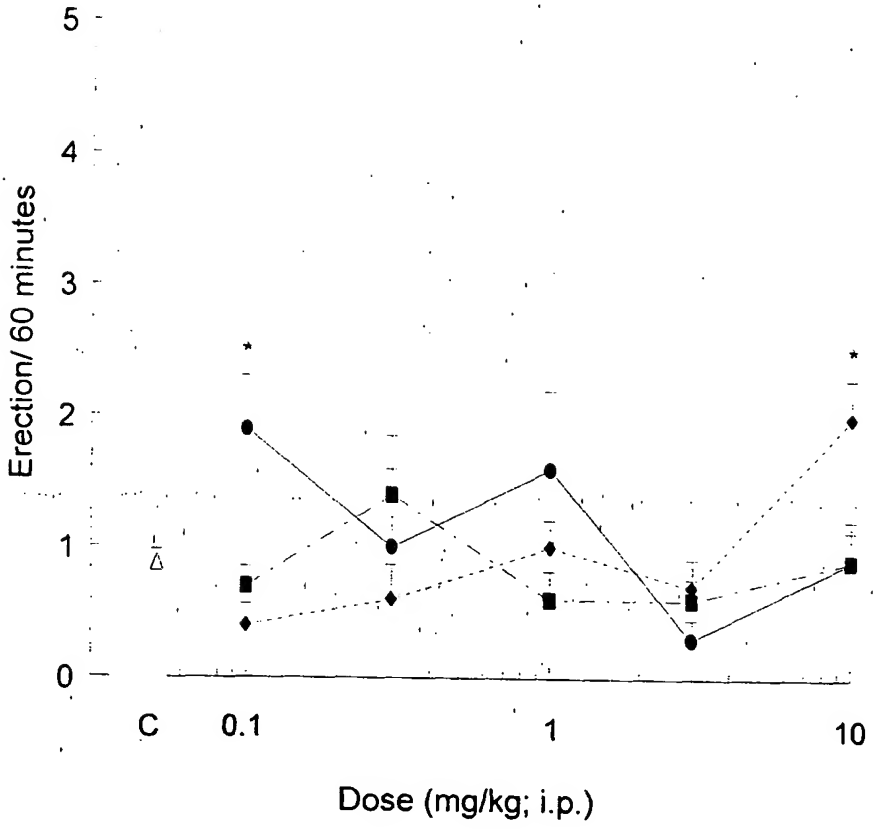


Figure 5

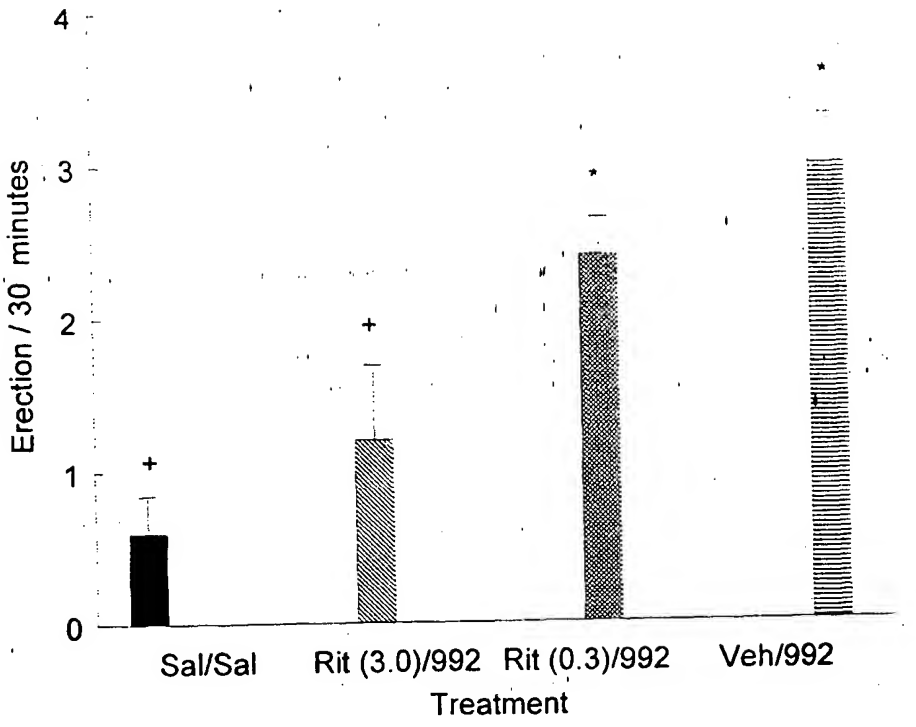

4
3
2
1
0
Erection / 30 minutes
Sal/Sal
Rit (3.0)/992
Rit (0.3)/992
Veh/992
Treatment

Figure 6

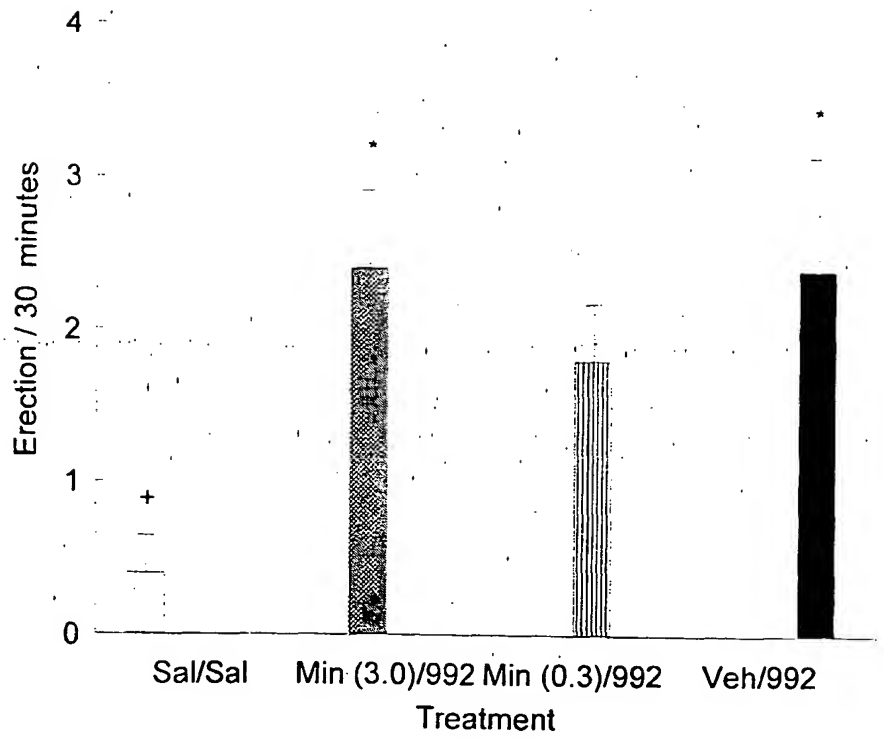

4
3
2
1
0
Erection / 30 minutes
Sal/Sal
Min (3.0)/992
Min (0.3)/992
Veh/992
Treatment

Figure 7

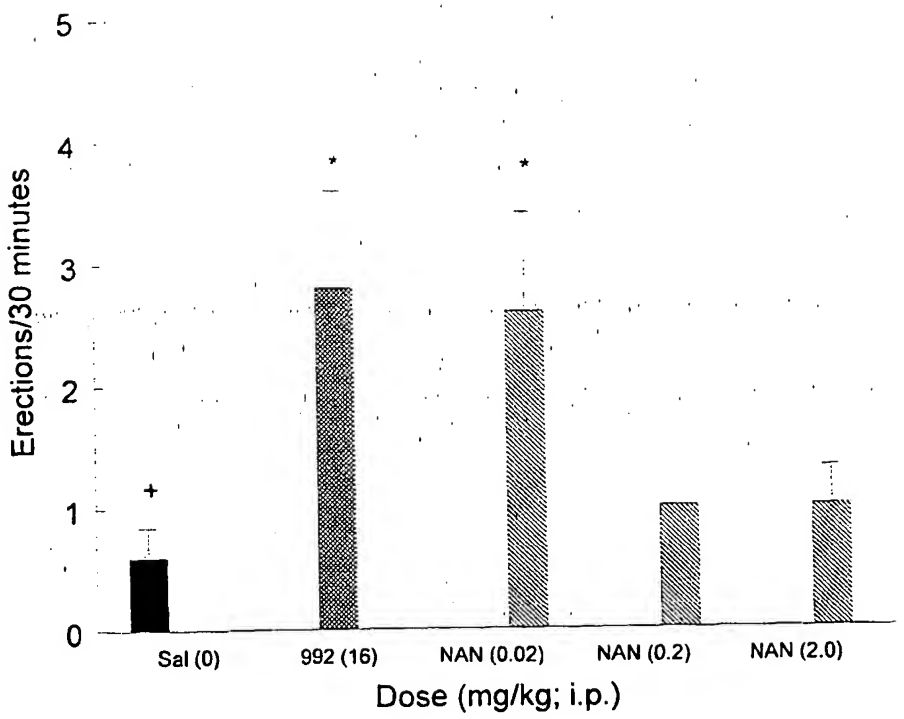

5
4
3
2
1
0
Erections/30 minutes
Sal (0)
992 (16)
NAN (0.02)
NAN (0.2)
NAN (2.0)
Dose (mg/kg; i.p.)

Figure 8

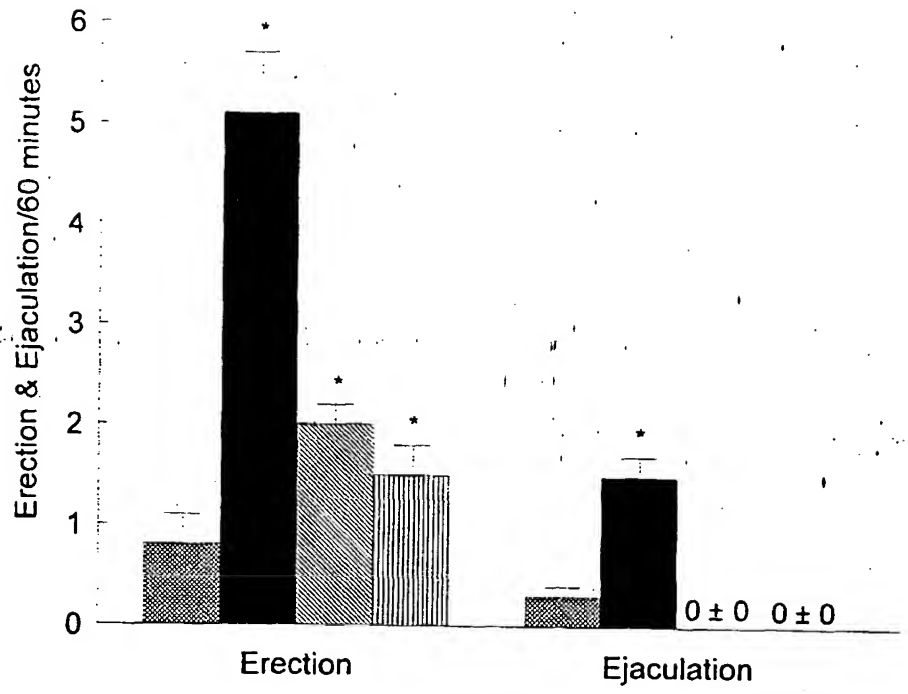

Erection & Ejaculation/60 minutes
6
5
4
3
2
1
0
*
*
*
*
0 ± 0
0 ± 0
Erection
Ejaculation

Figure 9

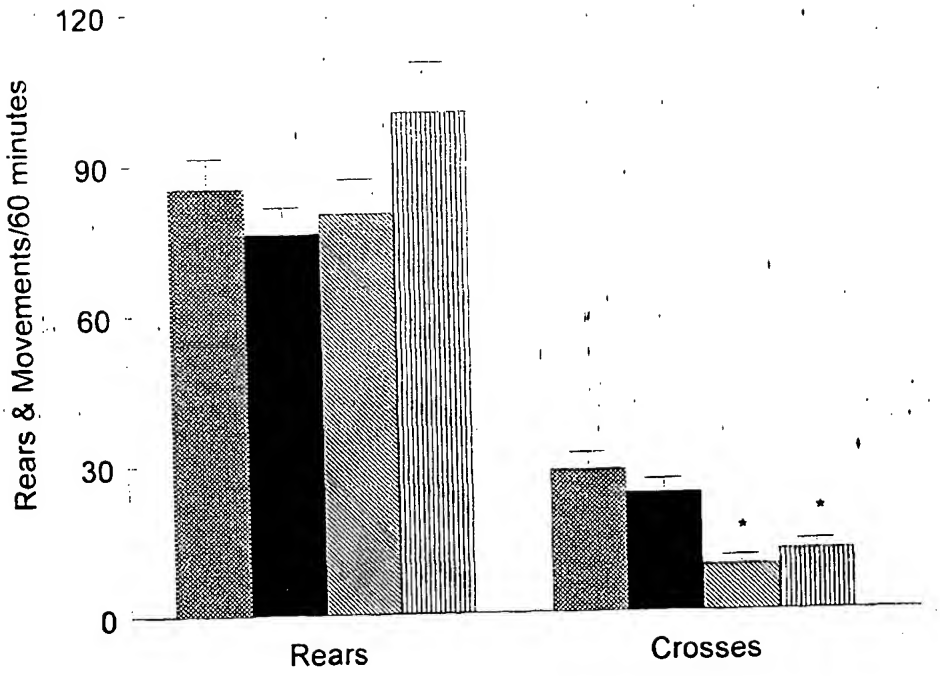


Figure 10

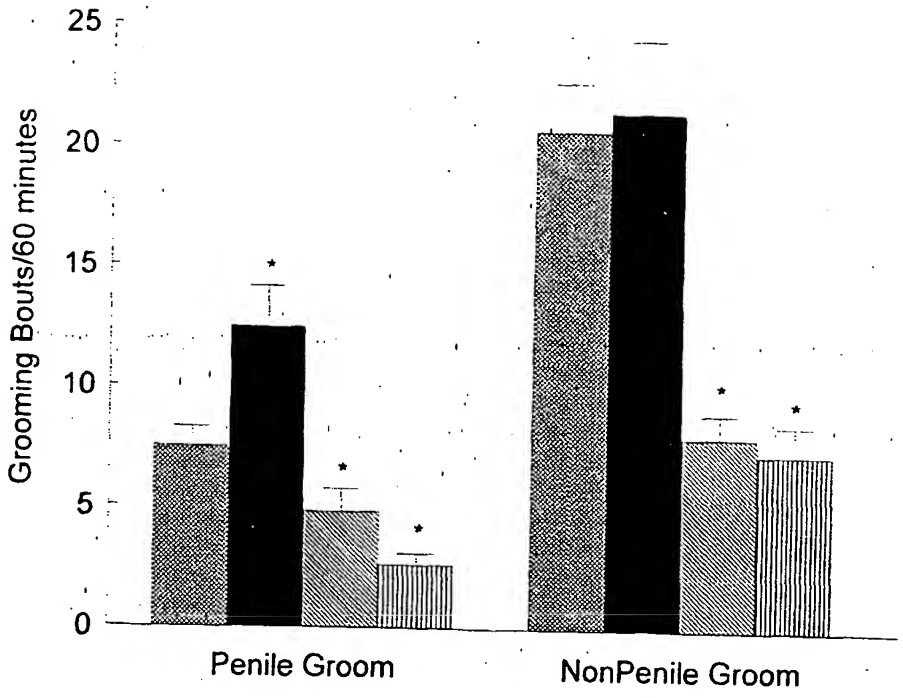

25
20
15
10
5
0
Grooming Bouts/60 minutes
Penile Groom
NonPenile Groom

Figure 11

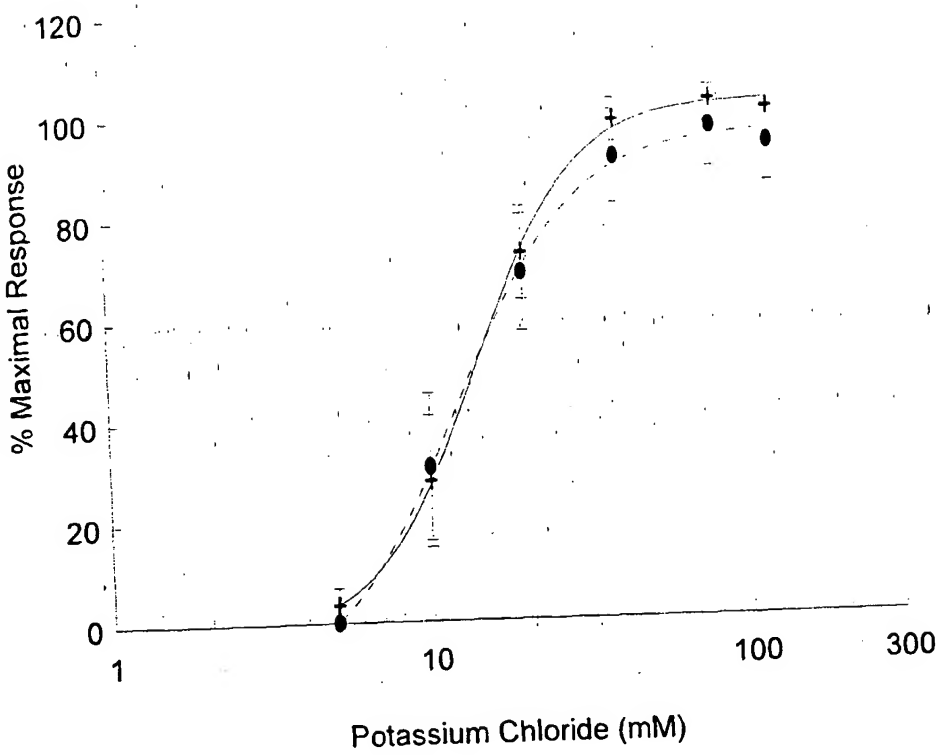

120
100
80
60
40
20
0
% Maximal Response
1
10
100
300
Potassium Chloride (mM)

Figure 12

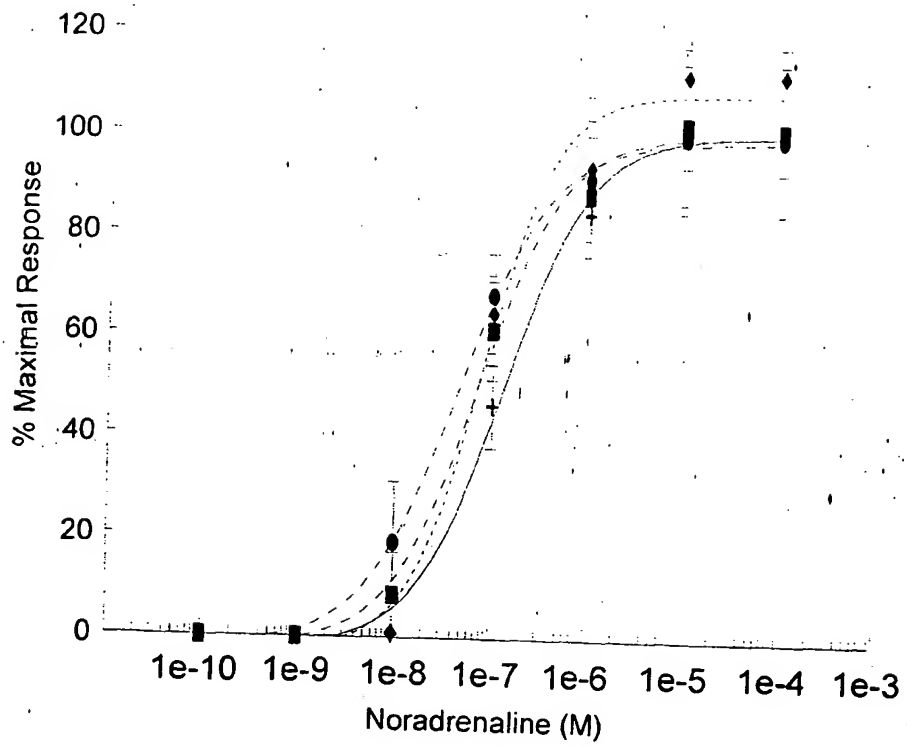

120
100
80
60
40
20
0
% Maximal Response
1e-10
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
1e-3
Noradrenaline (M)

Figure 13

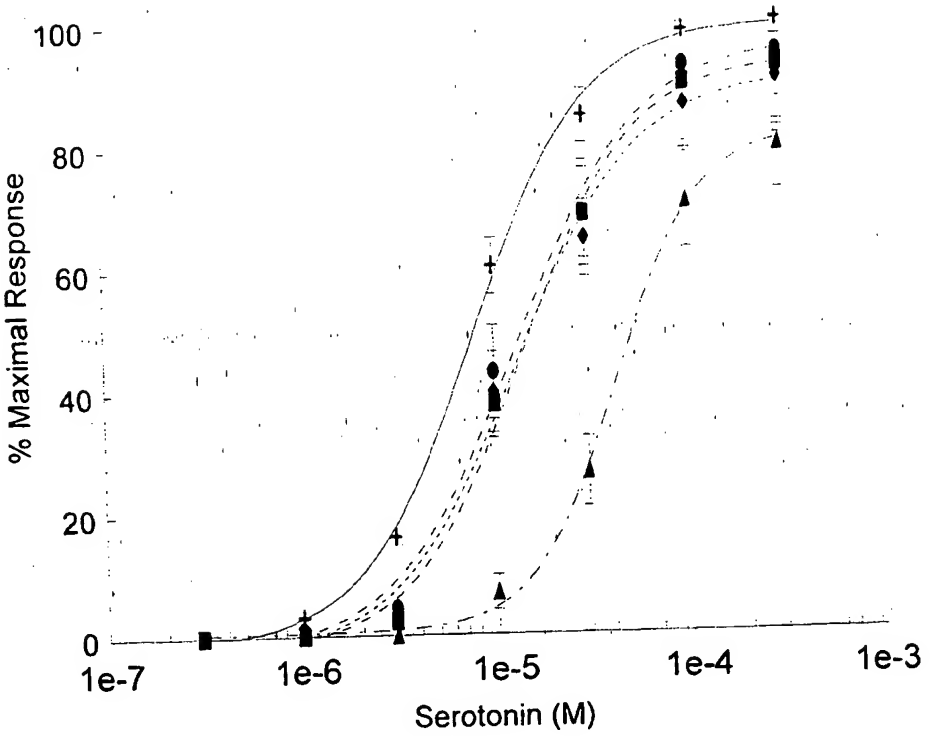

100
80
60
40
20
0
% Maximal Response
1e-7
1e-6
1e-5
1e-4
1e-3
Serotonin (M)

Figure 14

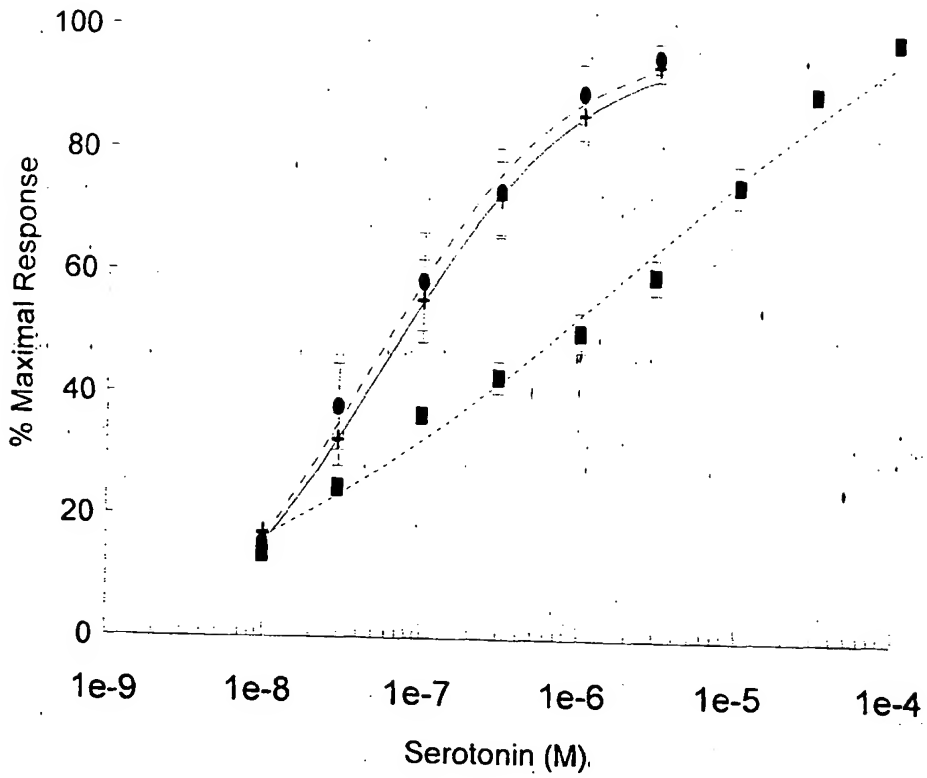

100
80
60
40
20
0
% Maximal Response
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
Serotonin (M)

Figure 15

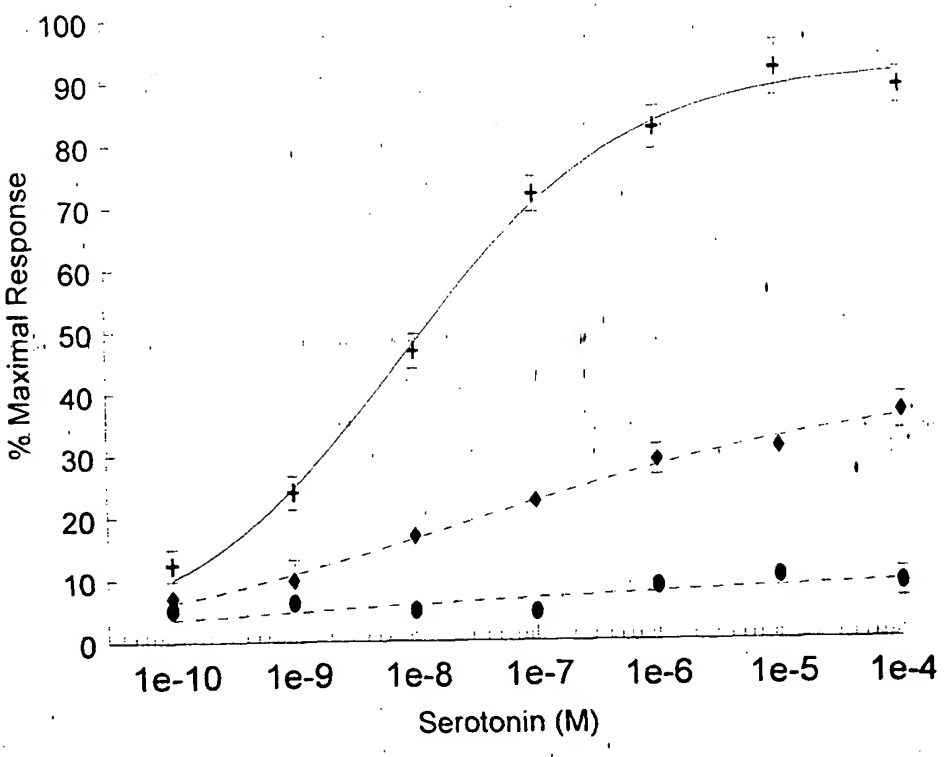

100
90
80
70
60
50
40
30
20
10
0
% Maximal Response
1e-10
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
Serotonin (M)

Figure 16

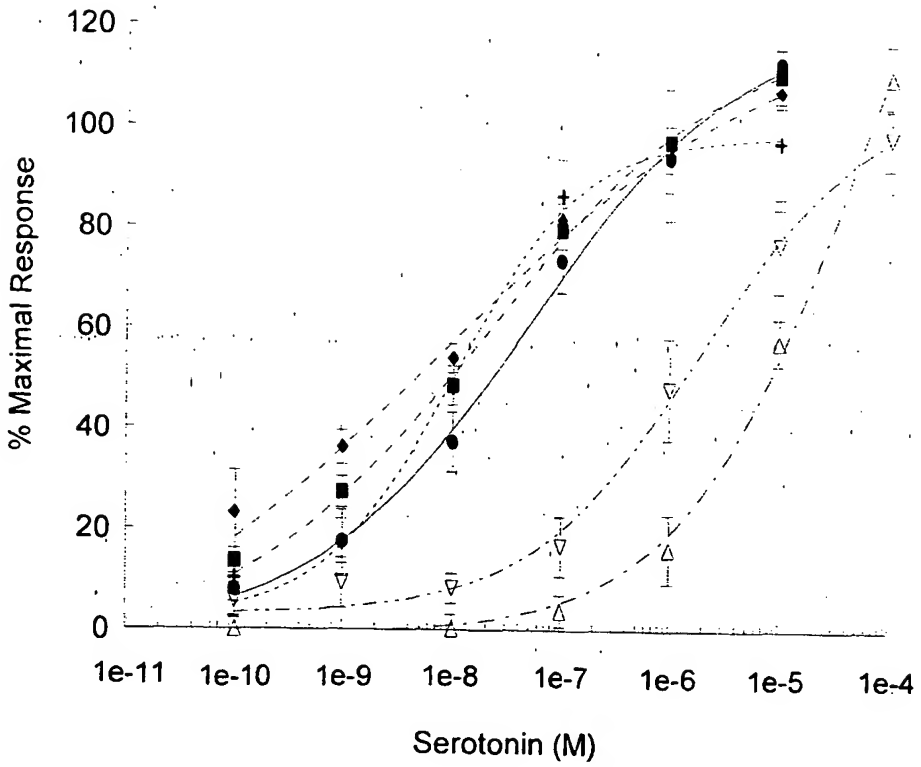

120
100
80
60
40
20
0
% Maximal Response
1e-11
1e-10
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
Serotonin (M)

Figure 17

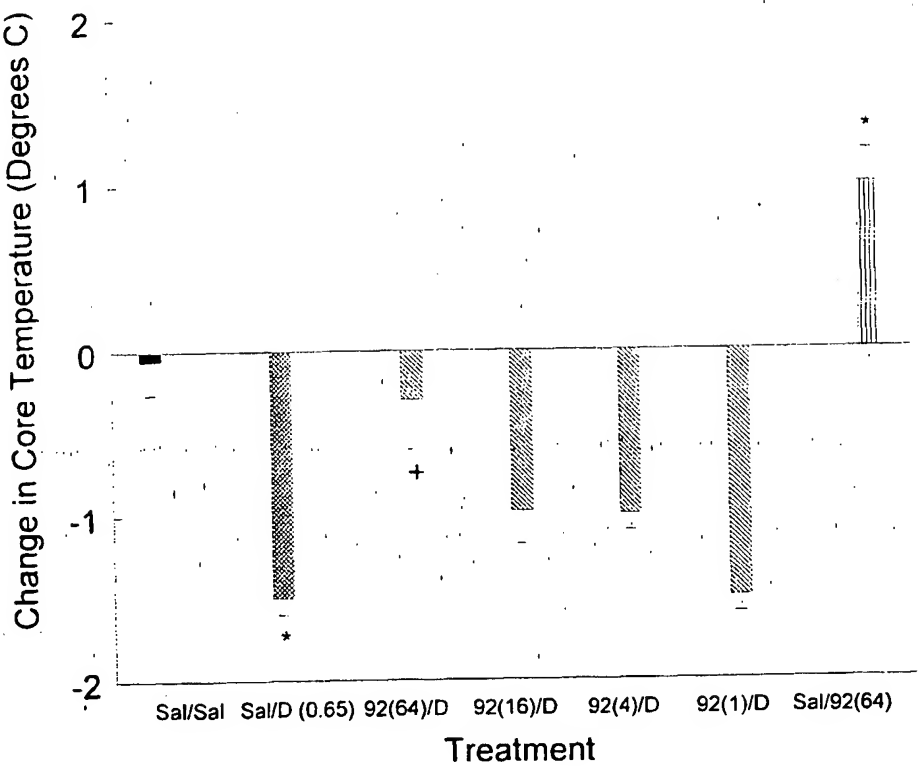

Change in Core Temperature (Degrees C)
2
1
0
-1
-2
Sal/Sal
Sal/D (0.65)
92(64)/D
92(16)/D
92(4)/D
92(1)/D
Sal/92(64)
Treatment

Figure 18

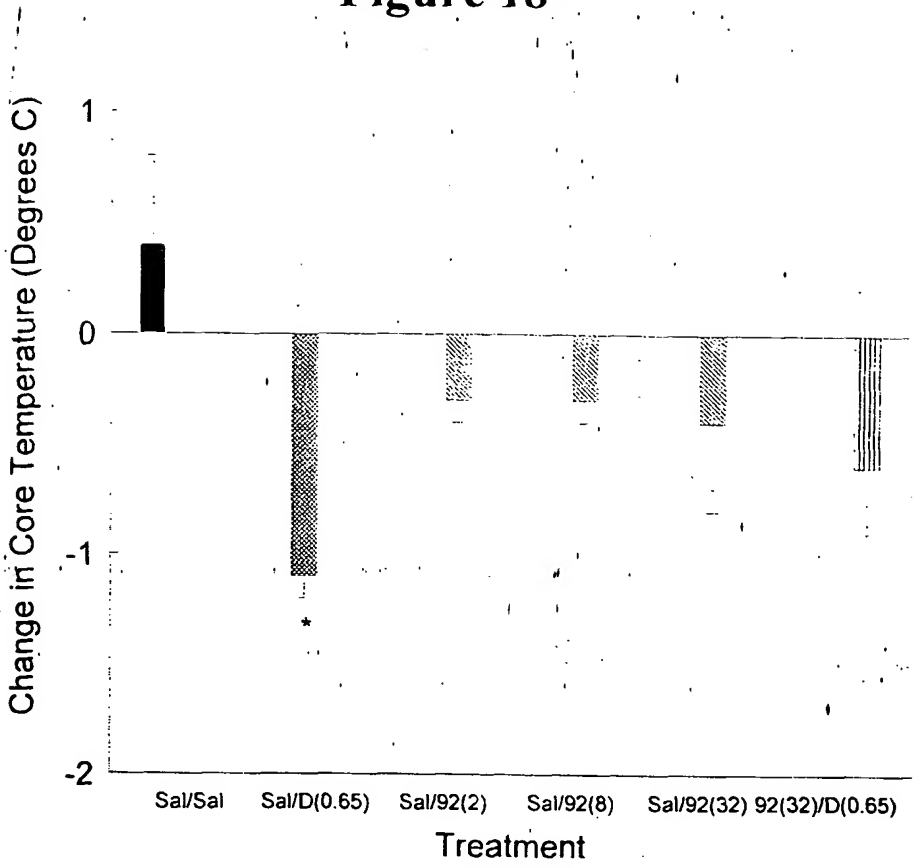

Change in Core Temperature (Degrees C)
1
0
-1
-2
*
Sal/Sal
Sal/D(0.65)
Sal/92(2)
Sal/92(8)
Sal/92(32)
92(32)/D(0.65)
Treatment

Figure 19

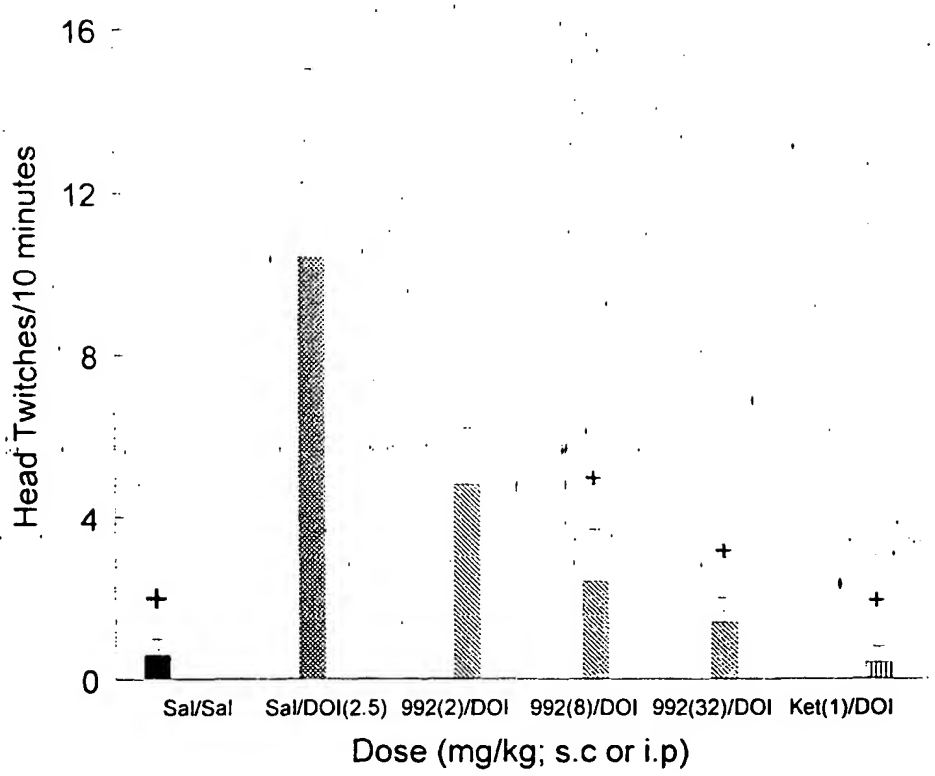
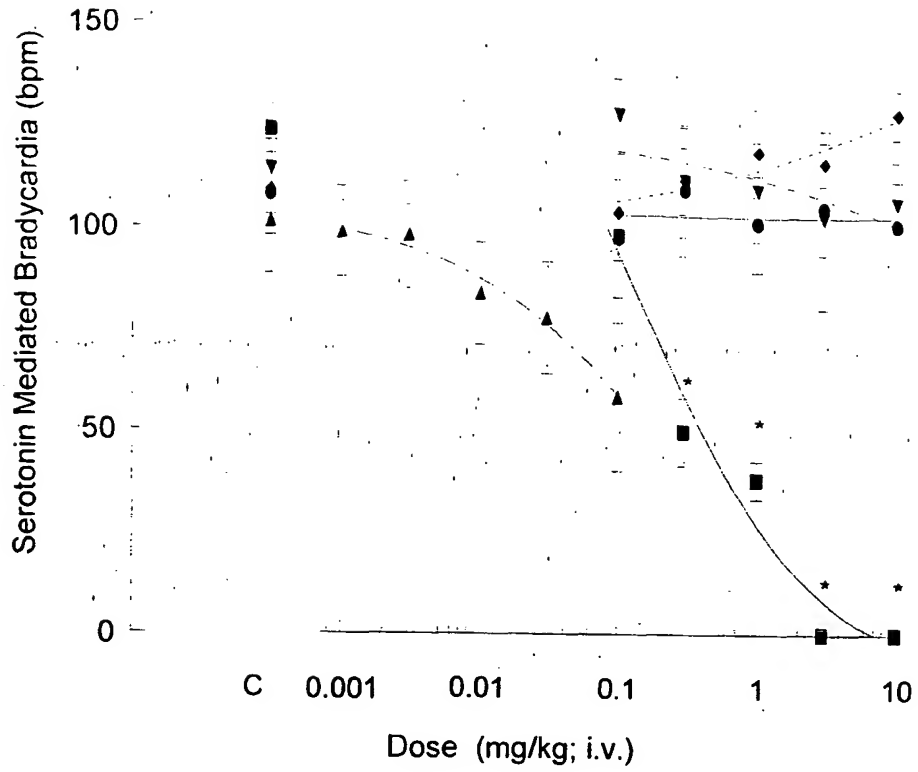

16
12
8
4
0
Head Twitches/10 minutes
Sal/Sal
Sal/DOI(2.5)
992(2)/DOI
992(8)/DOI
992(32)/DOI
Ket(1)/DOI
Dose (mg/kg; s.c or i.p)

Figure 20


Serotonin Mediated Bradycardia (bpm)
150
100
50
0
C
0.001
0.01
0.1
1
10
Dose (mg/kg; i.v.)

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 99/27484

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/495

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	"The pro-erectile project" NORTRAN PHARMACEUTICALS INTERNET SITE, 'Online! XP002132463 Retrieved from the Internet: <URL:http://www.nortran,com/projects/PE.html> 'retrieved on 2000-02-09! figure 1 —	1-79
X	HAYES E S ET AL: "ACTIONS OF ARYLPIPERAZINES ON CORPUS CAVERNOSUM SMOOTH MUSCLE IN VITRO" ASIA PACIFIC J. PHARMACOL., vol. 12, no. 3-4, 1997, pages 97-103, XP000874585 the whole document (RSD992) — -/--	1-79

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Orviz Diaz, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/US 99/27484

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E.S. HAYES ET AL.: "The effects of 5HT agonists on central peripheral and local erectile pathways" INT. J. IMPOT. RES., vol. 9, no. suppl., 1997, page s34 XP000878783 abstract A8	1-79
X	E.S. HAYES ET AL.: "RSD 992 enhances erection and copulation in rats and erection in primates" INT. J. IMPOT. RES., vol. 8, no. 3, 1996, page 189 XP000879354 abstract P24	1-79
E	WO 00 02550 A (NORTRAN PHARMACEUTICALS INC ;ZOLOTOY ALEXANDER B (CA); HAYES ERIC) 20 January 2000 (2000-01-20) the whole document	1-79
P,X	WO 99 02159 A (NORTRAN PHARMACEUTICALS INC ;ZOLOTOY ALEXANDER B (CA); HAYES ERIC) 21 January 1999 (1999-01-21) the whole document	1-79
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US FIORELLA D ET AL: "The role of the 5-HT2A and 5-HT2C receptors in the stimulus effects of m-chlorophenylpiperazine." retrieved from STN Database accession no. 95388786 XP002132520 abstract & PSYCHOPHARMACOLOGY, (1995 MAY) 119 (2) 222-30. ,	1-57, 66-71, 75-79
X	BERENDSEN H H G ET AL: "INVOLVEMENT OF 5-HT1C-RECEPTORS IN DRUG-INDUCED PENILE ERECTIONS INRATS" PSYCHOPHARMACOLOGY,DE,SPRINGER VERLAG, BERLIN, vol. 101, no. 1, 1 May 1990 (1990-05-01), pages 57-61, XP000673457 ISSN: 0033-3158 the whole document	1-57, 66-71, 75-79

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Intern. nal Application No
PCT/US 99/27484

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M BÖS: "Novel agonists of 5HT2c receptors. Synthesis and biological evaluation of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno'1,2-b!pyrrol-1-yl)-1-methylethyl amines. Improved therapeutics for obsessive compulsive disorder" JOURNAL OF MEDICINAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 40, no. 17, 15 August 1997 (1997-08-15), pages 2762-2769, XP002124091 ISSN: 0022-2623 page 2763, left-hand column, last paragraph; table 2	1-57, 66-71, 75-79
X	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US MILLAN M J ET AL: "5-HT2C receptors mediate penile erections in rats: actions of novel and selective agonists and antagonists." retrieved from STN Database accession no. 97296368 XP002132521 abstract & EUROPEAN JOURNAL OF PHARMACOLOGY, (1997 APR 23) 325 (1) 9-12. ,	1-57, 66-71, 75-79
A	DE COSTA, BRIAN R. ET AL: "Synthesis and evaluation of conformationally restricted N-'2-(3,4-dichlorophenyl)ethyl!-N-methyl-2 -(1- pyrrolidinyl)ethylamines at.sigma. receptors. 2. Piperazines, bicyclic amines, bridged bicyclic amines, and miscellaneous compounds" J. MED. CHEM. (1993), 36(16), 2311-20 , 1993, XP002132464 page 2312, scheme I, compounds 21, 25, 26	1-79
A	WO 95 08544 A (UNIV BRITISH COLUMBIA) 30 March 1995 (1995-03-30) examples 13,14 & US 5 637 583 A cited in the application	1-79

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/27484

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 31-35 and 69-74
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 31-35 and 69-74 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 99/27484

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0002550 A	20-01-2000	NONE	
WO 9902159 A	21-01-1999	AU 8203398 A	08-02-1999
WO 9508544 A	30-03-1995	AU 7650294 A	10-04-1995
		CA 2172513 A	30-03-1995
		EP 0720605 A	10-07-1996
		US 5637583 A	10-06-1997
		US 5885984 A	23-03-1999